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Descriptions of new species of Tertiary cycads, with a review of those previously recorded.[1]

ARTHUR HOLLICK

(WITH PLATES 1–14)

At the time when this paper was originally prepared for publication I received a copy of a similar one on the same general subject, from the author, Dr. Richard Kräusel of Frankfurt am Main.[2] The similarity between my contribution and the one by Doctor Kräusel, both in subject matter and in the manner of its presentation, may be regarded as a coincidence sufficiently interesting to be noted. I am pleased to hereby acknowledge my indebtedness to Doctor Kräusel for tracings, citations and other items of information in connection with certain Old World species, which otherwise would not have been in my possession.

DESCRIPTIONS OF NEW SPECIES

Genus CERATOZAMIA Brongniart

Ceratozamia Wrightii n. sp. (Plate 1). Leaf simply pinnate, dimensions not known. Leaflets entire, opposite or sub-opposite, normally subtending angles of about 80° with the rachis, linear-lanceolate to linear-elliptical, about 7.5 cm. in length by 1.2 cm. in maximum width, broadest below the middle, tapering distad to acute apices, more or less narrowed proximad to bases that terminate in triangular alate expansions through which they are attached to the sides of the rachis. Venation uniform, simple, parallel, the veins approximately 1 mm. or less apart.

Locality: Hamilton Bay, Kupreanof Island, Alexander Archipelago, Alaska.
Latitude: N. 57°.
Age and formation: Tertiary (Eocene) shaly sandstone.
Collector: C. W. Wright, U. S. Geological Survey, 1904.
Specimen in U. S. National Museum.

This species is, apparently, referable to the genus *Ceratozamia*, as far as may be inferred from the most striking of the surficial foliar characters that are discernible; and in order to facilitate comparison I have introduced (plate 2) a figure of a median portion of a leaf of the existing Mexican species *Ceratozamia mexicana* Brongniart, reduced to about one half

[1] Published with the permission of the Director of the United States Geological Survey.

[2] Paläobotanische Notizen IX. Über eine Cycadee aus dem Cerithienkalken von Nierstein und über die tertiäre Verbreitung der Cycadeen. Senckenbergiana **10:** 103–111. *pl. 1.* 1928.

[THE BULLETIN FOR MARCH (**59:** 109–168) WAS ISSUED 12 APRIL 1932.]

the natural size. The blunt apices of the leaflets might be regarded as more suggestive of the genus *Zamia;* but the relatively broad bases of the leaflets and the manner of their attachment to the rachis, are characteristic of *Ceratozamia* rather than of *Zamia.*

Our specimen is the first fossil representative of the genus thus far recorded from the New World; but a Tertiary (Miocene) species (*Ceratozamia Hofmanni*) from Switzerland, represented by a single parallel-veined leaflet, was described and figured by Ettingshausen; and a somewhat similar but more fragmentary specimen, from the Tertiary (Oligocene) of Italy, was described and figured by Meschinelli under the name *Ceratozamites vicetinus.* The relationship of each of these species to the Cycadaceae has, however, been questioned. The two species are listed and discussed on page 180 in this paper.

In our existing flora the northern limit of the range of the genus *Ceratozamia* is about latitude 25°, in Mexico, its only known region of distribution; but the discovery of our fossil species, in rocks of Tertiary age in Alaska, at about latitude 57°, represents presumptive evidence that tropical or sub-tropical climatic conditions prevailed there at that time. We may also regard it as significant that, as far as any records show, this Tertiary species was confined to the western American or Pacific region, as are the existing species of the genus, indicating that, even as far back as the early part of that period, milder climatic conditions prevailed in southeastern Alaska than in equivalent latitudes in the interior and in the eastern part of the continent.

The specific name is given in honor of Mr. C. W. Wright, by whom the specimen above described was collected.

Genus Dioön Lindley

Dioön inopinus n. sp. (Plate 3). Leaf pinnate. Leaflets linear-lanceolate or ligulate, slightly falcate, attached to the sides of the rachis, entire (sparingly denticulate above the middle?), 12.5 cm. or more, in length by 1.5 cm. in width near the middle, slightly contracted and then abruptly expanded at the bases, distinct and separate from each other throughout. Venation uniform, simple, parallel, the veins approximately .5 mm. apart.

Locality: South side, near the head of Hamilton Bay, Kupreanof Island, Alexander Archipelago, Alaska.

Latitude: N. 57°.

Age and formation: Tertiary (Eocene) shale.

Collector: W. W. Atwood, U. S. Geological Survey, 1907.

Specimen in U. S. National Museum.

This specimen, although fragmentary, indicates that the leaf, in its entirety, was apparently comparable in size with the average of the exist-

ing species of the genus. The leaflets, as far as discernible, are entire; but a single, well defined denticle, on the upper margin of the second lower leaflet on the left hand side of the specimen, may indicate that the distal parts of at least some of the leaflets were denticulate.

This species, and the one next described, are the first fossil representatives of the genus *Dioön* thus far recorded from any American locality, although several species have been described under the genus *Dioönites*, in particular from Mesozoic strata, among which it may be pertinent to refer to *Dioönites borealis* Dawson,[3] from the Cretaceous of the Northwest Territory, as a near ancestral relative of the two species of *Dioön* now under consideration, from the nearby coastal region of Alaska.

It is of interest to note, as was discussed in connection with the genus *Ceratozamia*, that the last representatives of the genus *Dioön* to survive in the northern part of North America were, apparently, confined to the Pacific coast region. At least no specimens that could be referred to the genus have been found anywhere in the interior or in the eastern part of the Continent. Until as recently as early Tertiary time, and perhaps later, their northern range extended to latitude 57° in Alaska, and possibly farther, whereas the present northern limit of distribution of the genus is about latitude 30° in Mexico, where it reaches, also, its furthest eastern distribution. These facts constitute important indices of the probable climatic conditions that prevailed in the region during the early part of the Tertiary period.

On the piece of matrix that contains our specimen may be seen numerous remains of *Sequoia Langsdorfii* (Brongniart) Heer, and fragments of a leaf that appear to represent a *Fagus*.

Dioön praespinulosum n. sp. (Plate 4, figs. 1, 2). Leaves simply pinnate or sub-pinnatifid. Leaflets ligulate, tapering distad, crowded, alternately arranged, as far as discernible, attached to the sides of the rachis by the entire extent of their bases, sparingly spinose-denticulate distad, 2–6 cm. or more in length by 5–8 mm. in maximum width. Venation uniform, simple, parallel, the veins approximately .5 mm. apart.

Locality: South side, near the head of Hamilton Bay, Kupreanof Island, Alexander Archipelago, Alaska.

Latitude: N. 57°.

Age and formation: Tertiary (Eocene) shale.

Collector: W. W. Atwood, U. S. Geological Survey, 1907.

Specimen in U. S. National Museum.

[3] Dawson, J. W. On the Cretaceous and Tertiary floras of British Columbia and the North-West Territory. Trans. Roy. Soc. Canada 1[4] [1882]: 24. *pl. 3, f. 37.* 1883.

This species is closely similar, in its general surficial appearance, to the existing Mexican species *Dioön spinulosum* Dyer, of which species a figure of a specimen (plate 5) is introduced for comparison. The general resemblance between them is unmistakable, and the minor characters may be seen to be strikingly alike.

The specimens figured on plate 4 show an upper part of a leaf (fig. 1) and a lower, median part (fig. 2). Other specimens, more or less dismembered and fragmentary, could have been selected to further illustrate the species; but the two figured should be sufficient for identification.

CYCAD (gen. et sp.?)

Locality: Kern River region, near Bakersfield, Kern County, California.
Latitude: N. 35° 25′
Geological horizon: Lower Miocene (Temblor beds).

The remains included in the above record are represented by apical parts of stumps or trunks, contained in and mostly replaced by a fine grained calcareous sandstone with numerous foraminifera, described as a marine deposit.

The specimens belong in the collection of the California Academy of Sciences. They were recently made known to me and were transmitted to me for examination through the courtesy of Dr. G. D. Hanna, Curator of the Department of Paleontology of the Academy. A cursory examination was sufficient to determine their relationship with the Cycadales; but their closer identification, if possible, was recognized as highly desirable, and I transmitted the specimens to Dr. G. R. Wieland, at Yale University, to whom I am indebted for their more critical examination. A complete report, I am advised, will be published elsewhere. Doctor Wieland stated that sections of the trunks failed to reveal plant structure of any kind. Their identity as cycadaceous remains, however, was confirmed, and relegation to the form-genus *Bucklandia* was suggested, with a specific name indicative of the locality or geologic formation. This would be in accord with the publication of *B. niersteinensis* Kräusel (see page 179) from the Miocene of Germany, and would be non-committal in regard to identity with any existing genus, or any fossil genus based upon foliar organs. Generic identity with certain of the Eocene cycads of Alaska and the existing cycads of Mexico may, however, be regarded as probable.

The locality where the specimens were found is indicated in its proper place on the map (plate 14). This locality record is of special interest as it represents a heretofore unknown but not unexpected fact in connection with the former distribution of the order.

PREVIOUSLY RECORDED SPECIES

New World—Northern Hemisphere

Genus ZAMIA Linnaeus

ZAMIA TENNESSEEANA Berry, U. S. Geol. Survey, Prof. Paper **156**: 51. *pl. 32. f. 8.* 1930. (Plate 13, Fig. 4)

Locality: West of Bolivar, Hardeman County, Tennessee.
Latitude: N. 35° 15′
Geological horizon: Lower Eocene (Wilcox group).

The author discusses the possible relationship of the species with the Cretaceous podocarpaceous genus *Nageiopsis*, but concludes that 'the probability is all in the direction of the present specimen representing a cycad pinnule'.

Figure 4, plate 13 is reproduced from a tracing of Berry's figure of the species, the description and illustration of which was published after our plates and figures had been arranged and numbered; hence it's inclusion, apparently out of place, with figures of Old World species, on plate 13.

As far as may be judged from an examination of the figure, the species appears to be, surficially, almost identical with small leaflets of the existing *Zamia angustifolia* Jacquin.

ZAMIA MISSISSIPPIENSIS Berry, Torreya **16**: 177. *f. 1–3.* 1916; U. S. Geol. Survey, Prof. Paper **108**: 63. *f. 17a, b, c.* 1917. (Plate 6, fig. 1 (a, b, c,))

Locality: Meridian, Lauderdale County, Mississippi.
Latitude: N. 32° 21′.
Geological horizon: Lower Eocene (Wilcox group).

This species was discussed by the author (*loc. cit.*) as suggestive of the existing *Zamia floridana* De Candolle.

ZAMIA (?) WILCOXENSIS Berry, U. S. Geol. Survey, Prof. Paper **91**: 169. *pl. 114, f. 2.* 1916. (Plate 6, fig. 6)

Locality: Four and a half miles southeast of Naborton, De Soto Parish, Louisiana.
Latitude: N. 32° 3′.
Geological horizon: Lower Eocene (Wilcox group).

This species was compared by the author (*loc. cit.*) with the existing *Zamia pumila* Linnaeus.

ZAMIA COLLAZOËNSIS Hollick, Sci. Surv. Porto Rico and Virgin Islands **7**[3]: 184. *pl. 53, f. 1, 3, 5, (7?).* 1928. (Plate 7, figs. 5, 6)

Locality: Collazo River, base of first falls below the Lares-San Sebastian Road bridge, Porto Rico.
Latitude: N. 18°.
Geological horizon: Upper Eocene or Oligocene.

Comparable with the existing *Zamia integrifolia* Jacquin, and *Z. salicina* Britton.

ZAMIA NOBLEI Hollick, Sci. Surv. Porto Rico and Virgin Islands **7**[3]: 185. *pl. 53, f. 9, 10; pl. 54, f. 1, 3a; pl. 55, f. 1–3, 4a, 5a.* 1928. (Plate 6, figs. 3, 4)

Locality: Collazo River, base of first falls below the Lares-San Sebastian Road bridge, Porto Rico.
Latitude: N. 18°.
Geological horizon: Upper Eocene or Oligocene.

Comparable with the existing *Zamia pumila* Linnaeus, *Z. umbrosa* Small, and *Z. integrifolia* Jacquin.

ZAMIA species, Berry, Proc. U. S. Natl. Mus. **75**[24]: 2. *pl. 1, f. 6.* 1929. (Plate 6, fig. 5)

Zamites species, *idem*, p. 12.

Locality: Montserrate, near Bogota, Colombia.
Latitude: N. 5°.
Geological horizon: Oligocene?

Figure 5 (=figure 6, Berry, *loc. cit.*) is depicted in a vertical position. The original figure is in a horizontal position, with the rounded end toward the right.

This species is represented by a ligulate fragment of a parallel-veined leaflet or pinnule which is abruptly contracted or rounded to a blunt termination at the one end that is preserved intact; but whether this is the proximal or the distal end can not be satisfactorily determined, although the latter is strongly suggested by the figure. The author, however (*loc. cit.*), described the specimen as 'contracted toward the base'. The author did not offer any suggestion in regard to comparison with or similarity to any existing species; but it may be seen to be very similar, in general appearance, to the distal parts of certain of the leaflets included under *Zamia Noblei*, as may be seen by comparison with our figures 3 and 4 on plate 6. Also it might be regarded as representing the distal extremity of a leaflet of which the proximal extremity was closely similar to the specimen represented by *Zamia wilcoxensis*, as may be seen by comparison with figure 6 on plate 6. In any event all three species, as far as may be judged by the figures, are suggestively similar in general appearance.

New World—Southern Hemisphere

Genus ZAMIA Linnaeus

ZAMIA PRAECEDENS Ettingshausen in Krasser, Sitzber. K. Akad. Wiss. Wien Math.-Naturw. Cl. **112**[1]: 853. 1903. [*Nom. nud.*]

Locality: Ouricanga (=Ouricanguinhas), about 25.5 km. northwest of the town of Alagoinhas, 14.7 km. north of Aramarý railroad station, Province of Bahia, Brazil.

Latitude: S. 12°.

Geological horizon: Late Tertiary (Pliocene?).

This species was not described or figured, and I have not seen the specimen. The author compared it with the existing *Zamia boliviana* De Candolle.

ZAMIA TERTIARIA Engelhardt, Abh. Senckenb. Naturforsch. Gesellsch. **16:** 646. *pl. 2, f. 16.* 1891; Berry, E. W. Johns Hopkins Univ. Stud. Geol. **4:** 120. *pl. 1* [misprinted 'VIII' in text], *f. 4; pl. 2,* [*f. 1–3,* not numbered on plate]. 1922. (Plate 7, figs. 1, [2, 3, 4?])

Locality: Coronel, Chile [Lota, Chile, *fide* Berry, *loc. cit.* p. 121], figs. 1, 4. Coranilahue, Chile, figs. 2, 3.

Latitude: S. 37°.

Geological horizon: Lower (?) Miocene.

Under the above specific name Berry (*loc. cit.*) includes large leaf forms (see our plate 7, figures 2, 3) very different, in size and shape, from *Zamia tertiaria* as described and figured by Engelhardt. He also relegates to the same species a parallel veined leaf which Engelhardt (*loc. cit.*, p. 686, pl. 1, fig. 4), somewhat doubtfully, designated 'Monokotyler Blattrest'. (see our plate 7, figure 4). Apparently there could be no serious objection to regarding Engelhardt's problematical monocotyledon as specifically identical with the *Zamia* leaflets figured by Berry; but their reference to *Z. tertiaria* as described and figured by Engelhardt (*loc. cit.*) appears to be open to question—judging by comparison between the figures. Under the circumstances the following quotation from Berry's discussion of the species (*loc. cit.*, p. 121) may be of interest:

> It is rather singular that with the small amount of material at his disposal Engelhardt should have recognized as representing a *Zamia* the small fragment figured by him and should have failed to recognize the relationship of the larger specimen which he refers to and figures as a fragment of a monocotyledonous leaf. This will account for the fact that Engelhardt compared the species with the existing *Zamia integrifolia* Ait. of Florida and the Antilles, when it actually is much more similar to several existing South American species.

It may also be of interest, in this connection, to compare Engelhardt's figure of *Z. tertiaria* (see our figure 1, plate 7) with *Z. collazoënsis* Hollick (fig. 5, plate 7), which latter was compared by me (Sci. Surv. Porto Rico **7**[3]**:** 184) with *Z. integrifolia* Jacquin; from which it might be inferred that the species last named (not *Z. integrifolia* Aiton) is the one that Engelhardt (*loc. cit.*, p. 620) intended to designate as resembling *Z. tertiaria*, in as much as the leaflets of *Z. integrifolia* Jacquin are relatively broad and

frequently short, whereas those of *Z. integrifolia* Aiton are relatively narrow and long. It may also be pertinent to note that in each of Engelhardt's figures the proximal and distal extremities are lacking, hence satisfactory comparison with them is impossible.

ZAMIA AUSTRALIS Berry, Proc. U. S. Natl. Mus. **73**[22]: 11. *pl. 2, f. 1.* 1928. (Plate 6, fig. 2)

Locality: Rio Nirihuao, near Casa Piedra, about 12 miles south of Lago Nahuel Huapi, Territory of Rio Negro, Argentina.
Latitude: S. 41°.
Geological horizon: Oligocene or lower Miocene.

The author did not compare this species with any now in existence. The fossil species which it resembles most closely—and the resemblance is remarkable— is *Zamites arcticus* Goeppert,[4] from the Lower Cretaceous (Komé) beds of Greenland.

Old World—Northern Hemisphere

Genus ZAMIPHYLLUM Caspary & Klebs

ZAMIPHYLLUM SAMBIENSE (Caspary) Caspary & Klebs, Abh. K. Preuss. Geol. Landesanst. n.s. **4**: 63 (text); *pl. 8, f. 51–51a* (atlas). 1907. (Plate 8, fig. 2)

Zamites sambiensis Caspary, Schrift. K. Physik.-Ökon. Gesell. Königsberg **22**: Sitzb. p. 26. 1882.

Locality: Samland, Baltic Prussia, Germany.
Latitude: N. 54° 50′.
Geological horizon: Middle Miocene (Tortonian?).

This genus and species is based upon two overlapping fragmentary leaflet remains, included in impure amber ('Braunharz' or 'Beckerit'), 13.5 mm. in length by 7 mm. in width, described (*loc. cit*, p. 26. 1882) as having 'etwa 35 parallelen dichten Nerven, die nach unten konvergiren, gegen die Spitze ganz parallel laufen und am Rande mit kleinem Bogen nach oben endigen'. The figure that serves to illustrate the species is enlarged to five times the natural size.

ZAMITES? PALAEOCENICUS Saporta & Marion, Mém. Cour. et Mém. Sav. Etrang. Acad. Roy. Sci. Belg. **41**[3]: 20. *pl. 1, f. 4, 5.* 1878. (Plate 8, fig. 3[4,5])

Zamites eocenicus Saporta & Marion. *op. cit.* p. 10 (*nom. nud.*).
Locality: Gelinden, Belgium.
Latitude: N. 50° 45′.
Geological horizon: Paleocene (Thanatian?).

[4] GOEPPERT, H. R. N. Jahrb. Mineral. Geol. u. Paläontol. **1866**: 134. *pl. 2, f. 9, 10.* 1866. HEER, OSWALD. Flora fossilis arctica. **1**: 82. *pl. 3, f. 14, 14b; pl. 44, f. 5c.* 1868.

This species is based upon two parallel-veined leaf fragments whose relationship with the Cycadaceae may, possibly, be regarded as open to question. As far as indicated by the figures the species might equally well be referred to the Monocotyledonae or, possibly, to a coniferous genus comparable with the existing *Dammara* (=*Agathis*) or *Podocarpus*; and in order to facilitate such comparison, I have introduced the accompanying text figures 1a, b, reproduced, respectively, from nature prints of leaves of *Dammara obtusa* Lindley and *Agagathis philippinensis Warburg*. Incidentally it may be of interest to compare all of the figures with those of *Zamia tertiaria* Engelhardt *fide* Berry. (See this paper. p. 175, pl. 7, figs. 2, 3.)

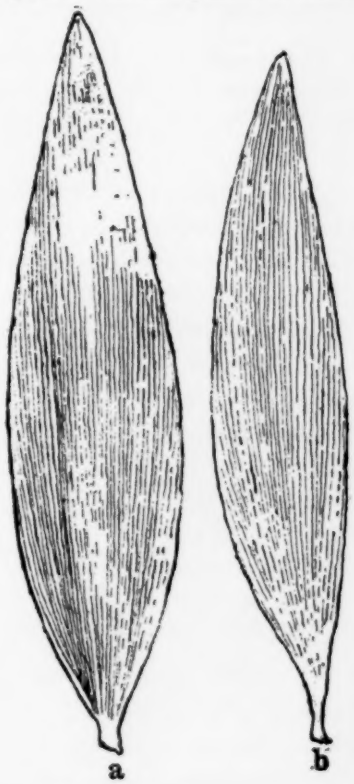

Fig. 1. a. *Dammara obtusa* Lindley. Specimen in conservatory, New York Botanical Garden. b. *Agathis philippinensis* Warburg. Luzon, March 25, 1904, *R. S. Williams 755;* specimen in herb. New York Botanical Garden.

ZAMITES (DIOÖN?) TERTIARIUS HERR, Flora Tertiaria Helvetiae **1:** 46. *pl. 16, f. 1 (a, b, c, d, e)*. 1855. (Plate 8, fig. 1)

Locality: Estavé, Vaud, Switzerland.
Latitude: N. 46° 30′.
Geological horizon: Miocene(Helvetian?).

This species is based upon what may be seen to be a poorly depicted specimen which, apparently, represents a fragmentary piece of a cycad leaf. The author discusses its probable relationship at some length, and finally concludes: 'Es gehört daher die fossile art wahrscheinlich zur Gattung Dion [Dioön]'. Schimper[5] suggested that 'c'est probablement un Dioön, voisin de notre *D. edule*'; and Kräusel[6] somewhat noncommittally remarked: 'Fiederbau und Nervatur sind mehr cycadeen- denn palmenähnlich'.

It would, obviously, be of little interest or value to here express any opinion in regard to the probable

[5] SCHIMPER, W. P. Traité de Paléontologie Végétale **2:** 157. 1870.
[6] KRÄUSEL, RICHARD. Senckenbergiana **10:** 106. 1928.

generic relationship of the species, in the absence of any personal examination of the specimen upon which the species was based.

ZAMITES EPIBIUS Saporta, Bull. Soc. Géol. France II. **21:** 322. *pl. 5, f. 1–3.* 1864 (=*Z. ephibius* in legend on plate 5); Ann. Sci. Nat. Bot. V. **8:** 10. *pl. 1, f. 1, 2.* 1867. (Plate 13, fig. $2^{1,2,3}$)

Locality: Bonnieux, Vaucluse (Provence), France.
Latitude: N. 43° 45′.
Geological horizon: Miocene (Aquitanian?).

Saporta's first description and illustration of this species (*loc. cit.*, 1864) included the leaf and two enlargements, reproduced in our plate 13, figure $2^{1,2,3}$; and his second (*loc. cit.*, 1867) included the leaf previously figured and a cone of more or less problematic botanical relationship. He (*loc. cit.*, pp. 10–11. 1867) compared the leaf with the Jurassic species *Zamia Feneonis* Brongniart[7] and *Z. formosus* Heer,[8] and the cone with female cones of the existing genus *Macrozamia*. In connection with these remains Kräusel[9] briefly comments that 'Das Blatt gehört einer Cycadee an; der damit vereinigte Zapfen ist aber wohl nur ein schlecht erhaltener, verdrückter Koniferenzapfen'.

As far as the surficial characters of the leaf and leaflets are concerned they might be regarded as indicative of relationship with *Encephalartus* rather than with *Zamia* (see *E. Gorceixianus*, plate 11).

ZAMITES RACZKIEVICZI Stur, Math. Termész. Köslem. Budapest **11:** 86. 1873. [nom. nud.]

Locality: Hungary?
Latitude: N. 46?
Geological horizon: Late Tertiary (Neogene)?

The above represents all of the information that I have been able to obtain in relation to this species. It was named, but apparently has been neither described nor figured.

Genus ZAMIOSTROBUS Endlicher

ZAMIOSTROBUS SAPORTANUS Schimper, Traité Paléont. Végétale **2:** 204. *pl. 72, f. 12.* 1870.

Locality: Armissan, Aude, France.
Latitude: N. 43°.
Geological horizon: Miocene (Aquitanian?).

[7] BRONGNIART, ADOLPHE. Dict. Sci. Nat. **57:** 99 (94). 1928 [*nom. nud.*]. *Zamites Feneonis* (Brongniart) Unger. Chloris Protogaea, pt. 6, p. lxiii. 1845. Ettingshausen. Abh. K. K. Geol. Reichsanst. 1^3: 9. *pl. 3, f. 1.* 1852.

[8] HEER, OSWALD. Urwelt der Schweiz, p. 144, *f. 94.* 1865.

[9] KRÄUSEL, RICHARD. Senckenbergiana **10:** 105. 1928.

This species was based upon a cycad strobile (female?), which was originally erroneously coupled by Schimper (*op. cit.*, p. 157) with the fossil leaf remains described and figured by Saporta under the name *Zamites epibius*,[10] but was subsequently determined not to belong with the remains representing that species, and was relegated to the form genus *Zamiostrobus*. Schimper's correction of the error (*op. cit.*, p. 204) is as follows:

C'est par erreur que j'ai dit, a l'occasion du *Zamites epibius*, que M. de Saporta rapporte le cône d'Armissan à cette feuille de Zamiée, rencontré à Orbagneux avec l'empreinte d'un cône mal conservé, dont la forme et la dimensions différent beaucoup du fruit dont il est question ici; ce dernier constitue évidement une espèce particulière que rapelle assez les cônes de certaines espèces de *Zamia* de l'époque actuelle.

Genus Bucklandia Presl

Bucklandia niersteinensis Kräusel, Senckenbergiana **10:** 107. *pl. 1, f. 1–3.* 1928.

Locality: Nierstein, Rhenish Hessen, Germany.
Latitude: N. 49° 45′.
Geological horizon: Miocene (Aquitanian?).

This species is based upon what appear to be well defined remains of cycadaceous trunks, which the author (*loc. cit.*, p. 108) refers to the form-genus *Bucklandia*, with the suggestion that, taking all circumstances and conditions into consideration, they might, tentatively, be referred to the existing African genus *Encephalartus*.

Genus Cycadites Sternberg

Cycadites Escheri Heer, Mitth. Naturf. Gesell. Zurich, **3:** 109. 1853 [*Nom. nud.*]; Flora Tertiaria Helvetiae **1:** 46. *pl. 15, f. 1, 2.* 1855.

Locality: Steinerweg ob Stein, Schaffhausen, Switzerland.
Latitude: 47° 45′.
Geological horizon: Miocene (Tortonian?).

This species is based upon remains of trunks that, as far as may be judged from the figures, simulate, more or less closely, those of a cycad. The author (*loc. cit.*) remarks upon their resemblance to *Endogenites echinatus* Brongniart,[11] but says:

Der Endogenites echinatus wird nicht mit Unrecht als Palmenstamm gedeutet, während der von Stein nicht von einer Palme herrühren kann, dagegen grosse Aehnlichkeit mit dem Stamme der Cycadeen hat.

I am not aware that any authority has ventured to refer the remains to any existing genus, and the opinion has been expressed that their defi-

[10] See this paper, p. 178, Pl. 13, fig. 2.
[11] Brongniart, A. T. Mém. Mus. d'Hist. Nat. [Paris] **8:** 301. *pl. 16 (5), f. 2.* 1822.

nite reference to the cycads is open to question. Any opinion based only upon examination and comparison of the several figures above cited would be of little value.

Genus CERATOZAMIA Brongniart

CERATOZAMIA HOFMANNI Ettingshausen, Sitzber. K. Akad. Wiss. Wien Math.-Naturw. Cl. **96**[1]: 80–81. 1887; Denkschr. K. Akad. Wiss. Wien Math.-Naturw. Cl. **54:** 272 [12]. *pl. 3, f. 10, (10a, 10b)*. 1888. (plate 13, fig. 1)

Locality: Leoben, Styria, Austria.
Latitude: N. 47° 30′.
Geological horizon: Miocene (Mayencian?).

This species is represented by a single falcate-lanceolate leaflet, which is more or less suggestive of *Zamia tertiaria* Engelhardt *fide* Berry,[12] as far as the shape is concerned (see our plate 7, figure 3). Comparison of other characters depicted in the figures would, however, be of little value. Study of the actual specimens would be necessary in order to arrive at satisfactory conclusions in regard to any further resemblances or any differences. It may be pertinent to suggest, however, that comparison with certain of the Monocotyledonae might not be out of place.

Genus CERATOZAMITES Meschinelli

CERATOZAMITES VICETINUS Meschinelli,[13] Atti Soc. Veneto-Trentina Sci. Nat. **10:** 276 [9], *pl. 6, figs. 1, 2*. 1889. (Plate 9, 10)

Locality: Monte Piano, Venetia, Italy.
Latitude: N. 45° 30′.
Geological horizon: Oligocene (Stampian?).

The author compared this species with the existing *Ceratozamia mexicana* Brongniart; but whether or not the comparison may be considered as justified will probably remain a matter of individual opinion. As far as is shown in the figures the general shape of the leaflets and their attachment to the rachis might, about equally well, be regarded as suggestive of relationship with the African genus *Encephalartus*, which reference would seem to be more logical, in view of the present geographical distribution of the two genera; but the rachis, it may be noted, is indicated as smooth and rounded, and is not strikingly suggestive of the upper or inner surface

[12] BERRY, E. W. Johns Hopkins Univ. Stud. Geol. 4: 120: *pl. 1, f. 4; pl. 2, f. 1–3*. 1922.

[13] Note. Writers subsequent to the date of Meschinelli's description of the species have written the specific name "*vicentinus*," which would seem to be the correct rendition. A.H.

of the rachis of a cycad leaf with attached leaflets; and in this connection it may be pertinent to cite Kräusel[14], who remarked, in his discussion of the apparent affinities of the species, that it 'könnte sehr wohl auch einer Palme angehören'.

Genus Encephalartus Lehmann

Plate 11

Encephalartus Gorceixianus Saporta, Comp. Rend. Acad. Sci. Paris **78:** 1320. 1874; La Nature **5**[2]**:** 262. *f. 9.* 1877; Soc. Bot. et Hort. Provence Bull. **2:** 42 (2). *pl.* [not numbered]. 1880.

Zamites Gorceixianus (Saporta) Renault, Cours de botanique fossile 1: 166 (tableau), *pl. 5, f. 1.* 1880.

Locality: Koumi, Island of Eubosa, Greece.
Latitude: N. 38° 20′.
Geological horizon: Miocene (Aquitanian?).

As far as I am aware no one has seriously questioned the validity of the reference of this species to the genus *Encephalartus*. Saporta (*loc. cit.*, pp. 43(3)–44 (4)) compared it with the existing *E. Altensteinii* Lehmann and an undescribed species from the northern interior of Africa, and remarked: 'Cette Cycadée fossile est d'autant plus intéressante qu'elle est la première qu'il ait été possible jusqu'ici d'inscrire sans anomalie dans un des genres vivants'.

Renault (*op. cit.*, p. 53) listed the species under Saporta's binomial, but included it, with other fossil species, under the genus *Zamites*, with the remark: 'Pour donner une idée de ce genre, nous choisirons les espèces suivantes', and then lists the species under consideration along with the Tertiary species *Zamites epibius* Saporta[15], and others of Mesozoic age.

Genus Cycas Linnaeus

Cycas fujiana Yokoyama, Jour. Coll. Sci. Imp. Univ. Tokyo **27**[20]**:** 4. *pl. 1, f. 7.* 1911. (Plate 12)

Locality: Manda, Miike coal field, Kiushiu, Japan.
Latitude: N. 33°.
Geological horizon: Eocene (Paleocene?).

The figure that serves to illustrate the description of this species may be seen to represent a cycad that is so closely similar to the existing *Cycas revoluta* Linnaeus as to be almost indistinguishable from it, as far as is indicated by form and general surficial features; and in this connection it is

[14] Kräusel, Richard. Senckenbergiana **10:** 105. 1928.
[15] See this paper, p. 178, Pl. 13, fig. 2.

of interest to note that *Cycas revoluta* is an element in the existing flora of Japan at about the same latitude as that in which the fossil species was found. Also this is the furthest north, in any part of the World, at which any existing species of the Cycadaceae is native.[16] There are none in Europe; and in North America the nearest approach is represented by the genus *Zamia* in northern Florida and *Dioön* in northern Mexico, at approximately latitude N. 31° and N. 30°, respectively.

Old World—Southern Hemisphere

Genus ANOMOZAMITES Schimper

ANOMOZAMITES MUELLERI Ettingshausen, Denkschr. K. Akad. Wiss. Wien Math. Naturw. Cl. **53:** 89. *pl. 8, f. 19–22.* 1887; translated by the author in Mem. Geol. Survey New South Wales (Paleontol. No. 2), p. 94. *pl. 8, f. 19–22.* 1888. (Plate 13, fig. 3^{19-22})

Locality: Vegetable Creek, near Emmaville, New South Wales, Australia.
Latitude: S. 29°.
Geological horizon: Lower (?) Eocene.

The five figures included in our figure 3, are tracings of the five figures of the specimens depicted by Ettingshausen (*loc. cit.*) to illustrate the description of this species. The numbers 19–22 are the figure numbers on his plate 8. Figure 20a is an enlarged representation of two contiguous pinnae or leaflets. The other figures represent specimens natural size. Figure 22 is described by the author as showing an early state of development of a leaf, and figures 19, 20 and 21 as distal and proximal remains of mature leaves.

The venation, as depicted, is that of a cycad, but the general appearance of the leaf may be regarded as somewhat suggestive of a fern; and Kräusel[17] remarked, in his discussion of the species; 'Ob nicht vielleicht doch eine Dicotyledone oder gar ein Farn vorliegt, könnte nur durch erneute Untersuchung des Originals aufgeklärt werden'.

Ettingshausen did not attempt any comparison with any existing or Tertiary cycad, but remarked (*loc. cit.*, p. 95) that 'one species, however, from the Cretaceous of North Greenland, described by Heer,[18] shows by

[16] I am indebted to Dr. Matajiro Yokoyama, of the Imperial University of Tokyo, for the following memorandum: "In the neighborhood of Tokyo *Cycas revoluta* is never seen wild, but south of it [35°] the plant is cultivated in gardens and thrives well. It is wild, perhaps, only in the southern part of Kyushu [Kiushiu]. Some tropical plants, such as *Livistonia* and *Ficus Wightiana* come up to the southern ends of Kyushu [31°] and Shikoku [33°]." A.H.

[17] KRÄUSEL, RICHARD. Senckenbergiana **10:** 105. 1928.

[18] Note. The only Cretaceous species of *Anomozamites* described by Heer from

its nerves, in some degree oblique to the rachis, a remarkable and specific relation to the fossil from the Eocene beds of Vegetable Creek.'

DISCUSSION

Tertiary cycads. The type of vegetation represented by the Cycadales or, to use a broader and more comprehensive term, the Cycadophyta, reached its maximum of development, biologically and numerically, in early Mesozoic time, during the Jura-Trias period, when it was, apparently, the most conspicuous and abundantly represented type in the World's vegetation. Remains of cycadophytes have been found in deposits of Triassic and Jurassic age in arctic and antarctic as well as in intermediate regions, indicating that during that time uniform tropical or subtropical climatic conditions prevailed throughout the World, from pole to pole.

At about the beginning of the Cretaceous period, coincident with the advent of the angiosperm type of vegetation, the cycadophytes began to wane numerically and to become more restricted in their distribution until, at the close of Mesozoic time, they represented a relatively unimportant element in the then existing flora.

In early Neozoic time cooler climatic conditions than had previously prevailed began to be clearly manifested in the polar regions. Climatic zones were definitely established. New types of vegetation were evolved, and any type that could not adapt itself to the changed conditions was either exterminated or was gradually driven further and further toward the equatorial region. The cycadophytes disappeared entirely as elements in the vegetation of the boreal and austral zones; and they made their last bid for existence in the temperate zones during Eocene and Miocene time, in certain regions or localities where favorable conditions still obtained. Any definite record of any Pliocene cycad north or south of the tropical zone is lacking. The advancing cold, that culminated in the wide-spread polar glaciation of the Quaternary period, and the contemporaneous increase in local glaciation elsewhere, ultimately resulted in the extermination of all species that could not migrate and join their relatives in the tropics.

In the New World well defined foliaceous remains of Tertiary cycads have been found, all apparently referable to the existing genera *Ceratozamia*, *Dioön*, and *Zamia*, and each in the region where it would be logical to expect it, in accordance with its existing area or range of distribution. The climatic conditions on the North American continent in early Terti-

Greenland, as far as I am aware, is *A. cretaceus* (Flora fossilis arctica 3^2: 70. *pl. 16, f. 19, 20.* 1874). This species, however, is represented by a small pinnatifid leaf or frond with forked veins. A.H.

ary (Eocene) time were apparently comparable, in certain respects, with those of today, as far as may be inferred from what records we have of the distribution of cycads at that time.

Any remains identified as those of cycads have not, as yet, been recorded from any Tertiary deposits in the north-central or eastern part of the North American continent; but from the early Eocene of the lower Mississippi valley, between latitudes 32° and 35°, three species of *Zamia* have been described, and from the Eocene of the Pacific Coast region in southeastern Alaska, at about latitude 57°, one species of *Ceratozamia* and two of *Dioön* are recognized. From these facts we may infer that the climate of the Pacific Coast at that time was milder than in the interior of the continent or eastward, as is the condition that obtains today.

Distribution of New World Tertiary cycads

CERATOZAMIA WRIGHTII n. sp.	Alaska. N.Lat.57°.
DIOÖN INOPINUS n. sp.	" " " "
D. PRAESPINULOSUM n. sp.	" " " "
CYCAD (gen. et. sp.?)	California. N.Lat.35°25′.
ZAMIA TENNESSEEANA Berry	Tennessee. N.Lat.35°15′
Z. MISSISSIPPIENSIS Berry	Mississippi. N.Lat.32°21′.
Z. (?) WILCOXENSIS Berry	Louisiana. N.Lat.32°3′.
Z. COLLAZOENSIS Hollick	Porto Rico. N.Lat.18°
Z. NOBLEI Hollick	Porto Rico. N.Lat.18°.
Z. species, Berry	Colombia. N.Lat.5°.
Z. PRAECEDENS Ettingshausen	Brazil. S.Lat.12°.
Z. TERTIARIA Engelhardt	Chile. S.Lat.37°.
Z. AUSTRALIS Berry	Argentina. S.Lat.41°.

The advancing cold, that culminated in the almost complete glaciation of the northern part of the continent in the Quaternary period drove the last remaining species of *Ceratozamia* and *Dioön* southward along the Pacific coastal region to where we now find the existing species of these genera, in Mexico. When and where they were last in existence in the intermediate region we have no available records; but we may be justified in assuming that they represented an element in the Miocene flora of the region, especially as cycads are known to have been in existence in Europe and possibly also in South America in what are now temperate latitudes, until about the middle of the Tertiary period.[19]

In the North American continental interior, and eastward, the cycads had been driven southward some time previously, to the Mississippi em-

[19] *Note.* After this was written I received from Dr. C. D. Hanna, Curator, Department of Paleontology, California Academy of Sciences, specimens that represent undoubted remains of cycads, recorded as Miocene in age, from Kern County, California (see page 172).

bayment, where at least three species of *Zamia* continued to exist until early Tertiary time. Apparently, however, conditions became more and more unfavorable. The limit of further southward migration had been reached and the genus was exterminated as an element in the continental flora of North America, until it subsequently returned by way of the Florida peninsula, where it's descendents now form a characteristic element in the existing flora.

Further southward, to the Equator and beyond, at least as far as south latitude 41°, some half a dozen specific representatives of the genus *Zamia* have been recorded as elements in the Tertiary floras of the Antillean region and South America. During that period, apparently, it was the dominant cycad genus of the New World, as it is at the present time.

In Europe the east-west direction of the principal mountain systems —the Pyrenees, Alps, Karpathians, etc.—and the Mediterranean Sea, formed impassable barriers to southward migration when driven in that direction for refuge from the constantly advancing cold of the Tertiary period.

A number of described species, based upon remains of leaves, fruit, trunks, etc. (described under the generic names *Zamites*, *Zamiphyllum*, *Zamiostrobus*, *Ceratozamites*, *Cycadites*, etc., in accordance with their apparent generic relationships), all more or less similar in surficial characters to similar parts in existing genera, were able to maintain their existence in Europe until about the middle of the Tertiary period. Their last known stand in the western part of the continent was made in the south of France, where the Pyrenees Mountains and the Mediterranean Sea barred them to the south, and these generic types were there exterminated. Further eastward the Alps, Karpathians, Balkans, and Caucasus Mountains formed equally impassable barriers, and there also the extermination was complete. In Greece, however, further to the south, a species of *Encephalartus* continued to exist for a while, after its other European relatives had perished. There was, apparently, more direct land connection at that time than now, between Greece and Africa, and although the genus finally became extinct in Europe, by reason of general climatic change, it had in the meantime become established southward, where its representatives compose the most extensive of the two existing cycad genera native in Africa. This genus may be regarded as satisfactorily identified in connection with the fossil species of Greece; but the generic affiliations of most of the other described European Tertiary spêcies may be regarded as not satisfactorily determined and, in connection with certain of them, relationship with the cycads in general appears to be open to question.

In Asia only one discovery of a Tertiary cycad has thus far been re-

corded—a well defined species of *Cycas*, in Japan. If any others were in existence at that time in the interior of the continent we may infer that they met with a fate similar to that which befel their contemporary relatives in Europe, by reason of similar east-west trends of many of the mountain ranges. The north-eastern part of the Asiatic continent, however, in common with the northwestern part of the North American continent, was not subjected to glaciation such as that which prevailed in Europe and in northern and eastern North America during the Quaternary period, although evidences of severe cold are manifest. Along the eastern coast, however, through Japan, China and the Malay peninsula a route for southern migration was open, of which representatives of the genus *Cycas* took advantage and subsequently returned by it to southern Japan when favorable climatic conditions were again established.

The only recorded Tertiary cycad from south of the Equator in the Old World is the more or less problematic *Anomozamites Muelleri*, described by Ettingshausen, from Australia. If this is a cycad it may represent an insular generic type, now extinct, which can not be satisfactorily compared with any now in existence.

Distribution of Old World Tertiary cycads

Zamiphyllum sambiense (Caspary) Caspary & Klebs	Germany. N.Lat.54°50′.
Zamites paleocenicus Saporta & Marion	Belgium. N.Lat.50°45′.
Z. (Dioön) tertiarus Heer	Switzerland. N.Lat.46°30′
Z. Raczkieviczii Stur	Hungary? N.Lat.46°?
Z. epibius Saporta	France. N.Lat.43°45′.
Zamoiostrobus Saportanus Schimper	France. N.Lat.43°.
Bucklandia niersteinensis Kräusel	Germany. N.Lat.49°45′.
Cycadites? Escherii Heer	Switzerland. N.Lat.47°45′.
Ceratozamia Hofmanni Ettingshausen	Austria. N.Lat.47°30′.
Ceratozamites vicetinus Meschinelli	Italy. N.Lat.45°30′.
Encephalartus Gorceixianus Saporta	Greece. N.Lat.38°20′.
Cycas fujiiana Yokoyama	Japan. N.Lat.33°.
Anomozamites Muelleri Ettingshausen	Australia. S. Lat.29°.

Existing cycads. The existing cycad flora is included in nine genera. The dominant genus in the New World is *Zamia*, with about thirty species and with a geographic range that extends from N. Lat. 31° to S. Lat. 22°. The outstanding genus in the Old World is *Cycas*, with about twenty species and a range that extends from N. Lat. 35° to S. Lat. 35°. These two are the only genera that cross the Equator and are common to both the Northern and the Southern Hemisphere. No genus of the Cycadaceae is common to both the Eastern and the Western Hemisphere. All of the genera are tropical or subtropical in their distribution.

The salient facts in connection with the distribution of the nine recognized genera may be tabulated as follows:

Genera	*Number of species*	*Distribution*	*Latitude (approximate)*
ZAMIA L.	±30	Florida, West Indies, Central and South America	N.31°–S.22°
DIOÖN Lindl.	± 4	Mexico	N.30°–N.17°
CERATOZAMIA Brongt.	± 6	Mexico	N.25°–N.17°
MICROCYCAS A. DC.	1	Cuba	N.22°
BOWENIA Hook. f.	2	Northeastern Australia	S.25°–S.15°
MACROZAMIA Miq.	±15	Australia	S.35°–S.15°
ENCEPHALARTUS *fide* Steud. (=ENCEPHALARTOS Lehm.)	±15	South Africa	S.35°–S.20°
STANGERA *fide* Voss (=STANGERIA T. Moore)	1	South Africa	S.32°–S.23°
CYCAS L.	±20	Japan, southern China, East Indies, Australia, islands of the Pacific and Indian Ocean (and east Africa?).	N.35°–S.35°

With the exception of *Zamia* and *Cycas* it may be noted that the genera are remarkably local and restricted in their distribution. *Dioön* and *Ceratozamia* are confined to Mexico; *Microcycas* to Cuba; *Bowenia* and *Macrozamia* to limited regions in Australia; *Encephalartus* and *Stangera* to South Africa. Furthermore they do not occur as pure stands or extensive aggregates but only in scattered groups or as individuals. Incidentally, also, two of the genera—*Microcycas* and *Stangera*—are monotypic, and one other—*Bowenia*—is represented by but two species. These three genera are obviously on the verge of extinction, and all of the others, with the exception of *Zamia* and *Cycas*, are so local and restricted in their distribution that a relatively slight environmental change, whether brought about through geologic or through human agency would, probably, result in their extermination.

SUMMARY AND CONCLUSIONS

Tertiary cycads are of special interest as representing the last bid for life of the cycadophytes outside of the tropical and subtropical zones as now recognized; they are identical or closely related generically to those now in existence in the tropics and subtropics; the close similarity between the fossil and the existing forms indicate that tropical or subtropical conditions prevailed in Tertiary time in the regions where the fossil forms are found; the existing cycadophytes represent the remnants of

a formerly widely distributed type of vegetation which may be regarded as approaching extinction, due, primarily, to unfavorable environmental and climatic changes over extensive areas of former occupation.

THE NEW YORK BOTANICAL GARDEN

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PLATE 2

Ceratozamia mexicana Brongniart (about one half natural size). Mexico. Specimen in Herb. New York Botanical Garden. Introduced for comparison.... 169

PLATE 3

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PLATE 4

Dioön praespinulosum n. sp. Kupreanof Island, Alaska. Fig. 1, apical portion of a leaf. Fig. 2, median portion 171

PLATE 5

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PLATE 6

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Fig. 2. *Zamia australis* Berry, Proc. U. S. Natl. Mus. **73**[22]**:** *pl. 2, fig. 1.* 1928.... 176

Figs. 3, 4. *Zamia Noblei* Hollick, Sci. Surv. Porto Rico and Virgin Islands vol. 7, pt. 3. 1928. Fig. 3 = *loc. cit. pl. 53, fig. 9.* Fig. 4 = *loc. cit. pl. 54, fig. 3a* 174

Fig. 5. *Zamia* species, Berry, Proc. U. S. Natl. Mus. **75**[24]**:** *pl. 1, fig. 6.* 1929..... 174

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PLATE 7

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Figs. 2, 3. *Zamia tertiaria* Engelhardt, *fide* Berry, Johns Hopkins Univ. Stud. Geol. No. 4. 1922. Fig. 2 = *loc. cit. pl. 1,* [misprinted 'VIII' in text] *fig. 4.* Fig. 3 = *loc. cit. pl. 2, fig. 3* [not numbered on plate] 175

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PLATE 8

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Fig. 1. *Zamites* (*Dioön*) *tertiarius* Heer, Flora Tertiaria Helvetiae **1:** *pl. 16, fig. 1.* 1855 177

Fig. 2. *Zamiphyllum sambiense* (Caspary) Caspary & Klebs (five times natural size), Abh. K. Preuss. Geol. Landesanst. n. s. No. 4, *pl. 8* (atlas), *fig. 51.* 1907. 176

Fig. $3^{4,5}$. *Zamites? palaeocenicus* Saporta & Marion, Mém. Cour. et Mém. Sav. Etrang. Acad. Roy. Sci. Belg. **41**[3]: *pl. 1, figs. 4, 5.* 1878. Fig. 3^4 = *loc. cit. fig. 4* (median portion of a leaflet). Fig. 3^5 = *loc. cit. fig. 5* (apical portion of a leaflet) 176

PLATE 9

Ceratozamites vicetinus Meschinelli, Atti Soc. Veneto-Trentina Sci. Nat. **10:** *pl. 6, fig. 1.* 1889 180

PLATE 10

Ceratozamites vicetinus Meschinelli, Atti Soc. Veneto-Trentina Sci. Nat. **10:** *pl. 6, fig. 2.* 1889 180

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Encephalartus Gorceixianus Saporta, Soc. Bot. et Hort. Provence, Bull. No. 2, *pl.* [not numbered]. 1880 181

PLATE 12

Cycas Fujiiana Yokoyama, Jour. College Sci. Imp. Univ. Tokyo **27**[20]: *pl. 1. fig. 7.* 1911 181

PLATE 13

Fig. 1. *Ceratozamia Hofmanni* Ettingshausen, Denkschr. K. Akad. Wiss. Wien, Math.-Naturw. Cl. **54:** *pl. 3, fig. 10.* 1888 180

Fig. $2^{1,2,3}$. *Zamites epibius* Saporta, Bull., Soc. Géol. France, II. **21:** *pl. 5, figs. 1–3.* 1864. Fig. 2^1 = *loc. cit., fig. 1* (leaf, natural size). Fig. 2^2 = *loc. cit., fig. 2* (leaflet, enlarged). Fig. 2^3 = *loc. cit., fig. 3* (median part of leaf, enlarged) 178

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Fig. 4. *Zamia tennesseana* Berry, U. S. Geol. Survey Prof. Paper **156:** *pl. 32, f. 8.* 1930 173

PLATE 14

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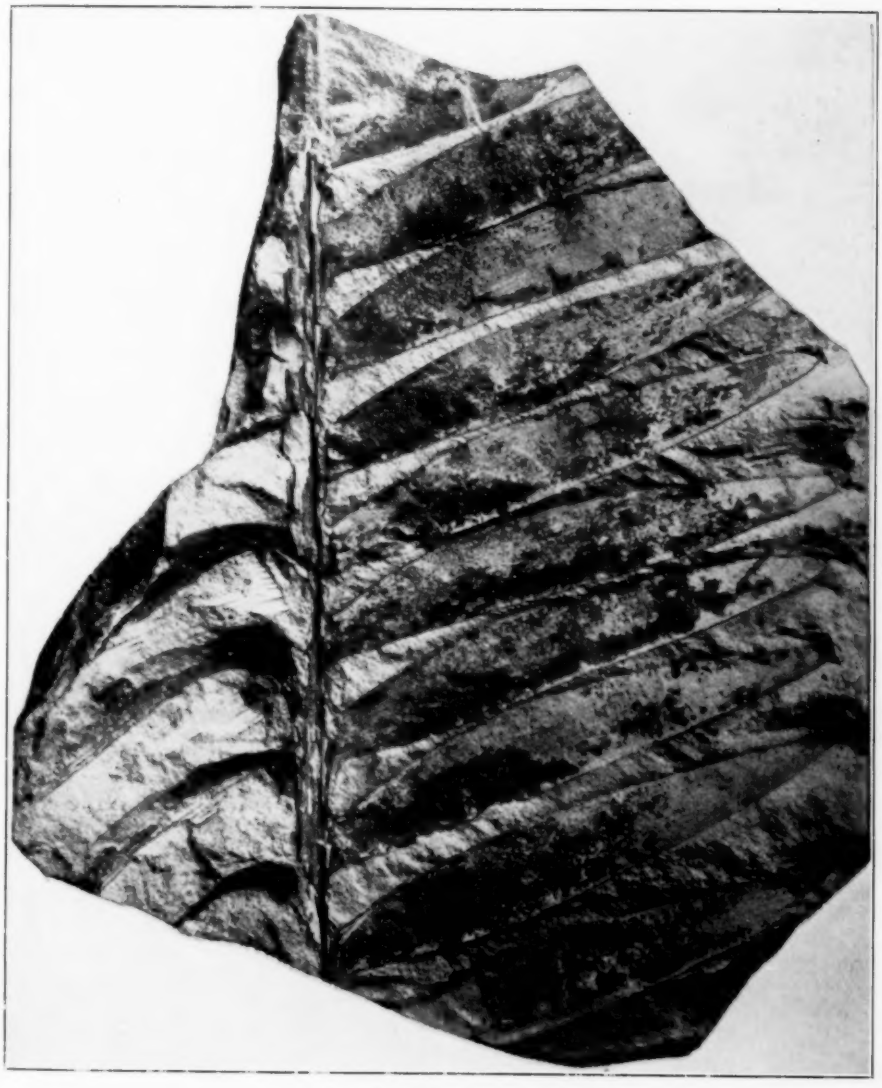
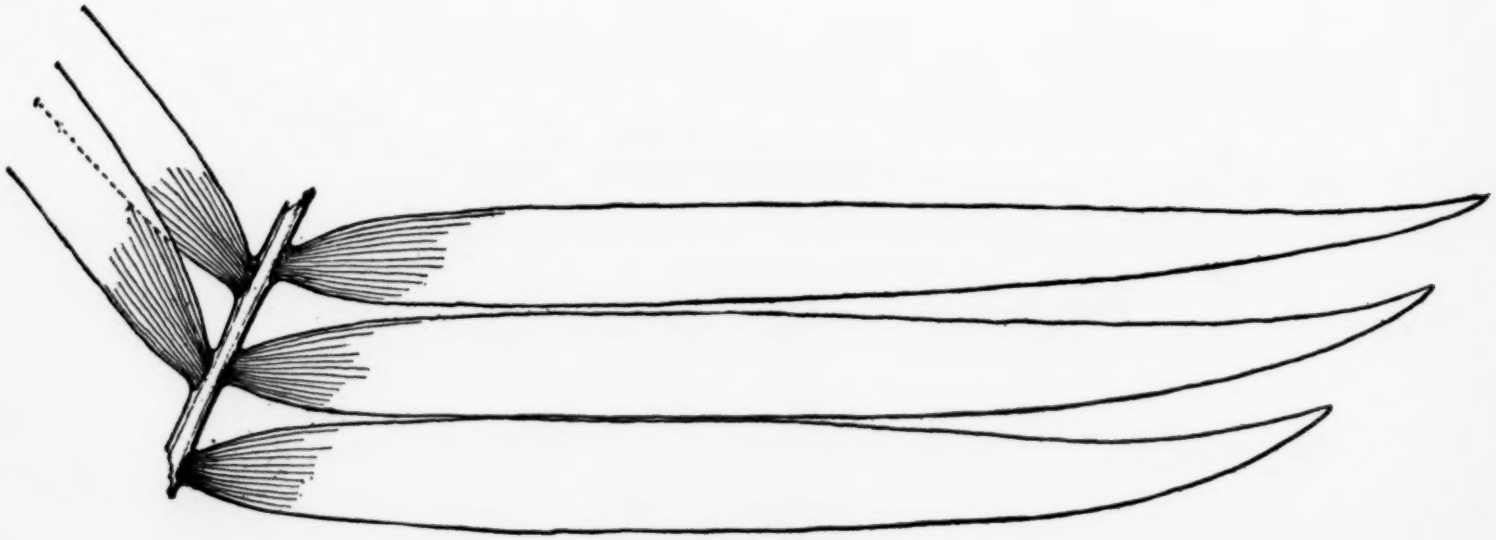


HOLLICK: TERTIARY CYCADS



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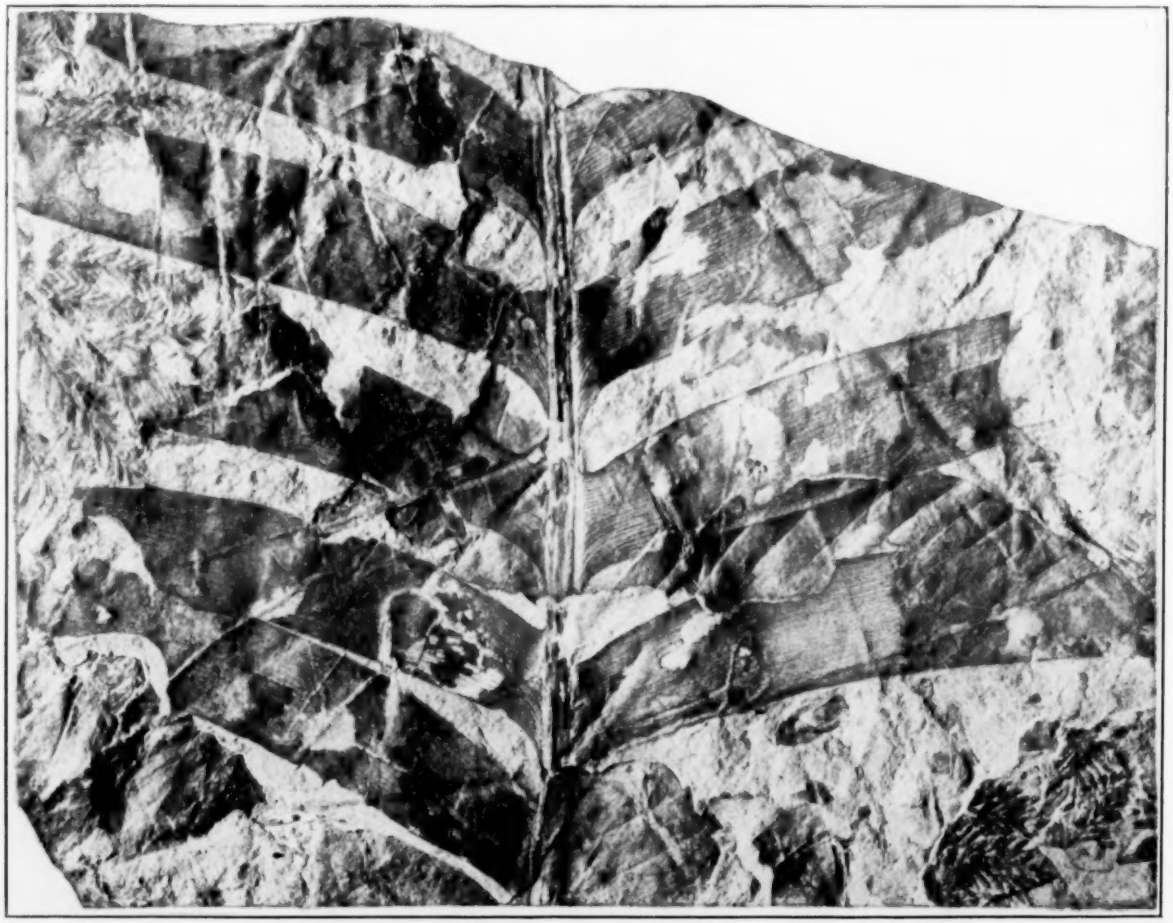

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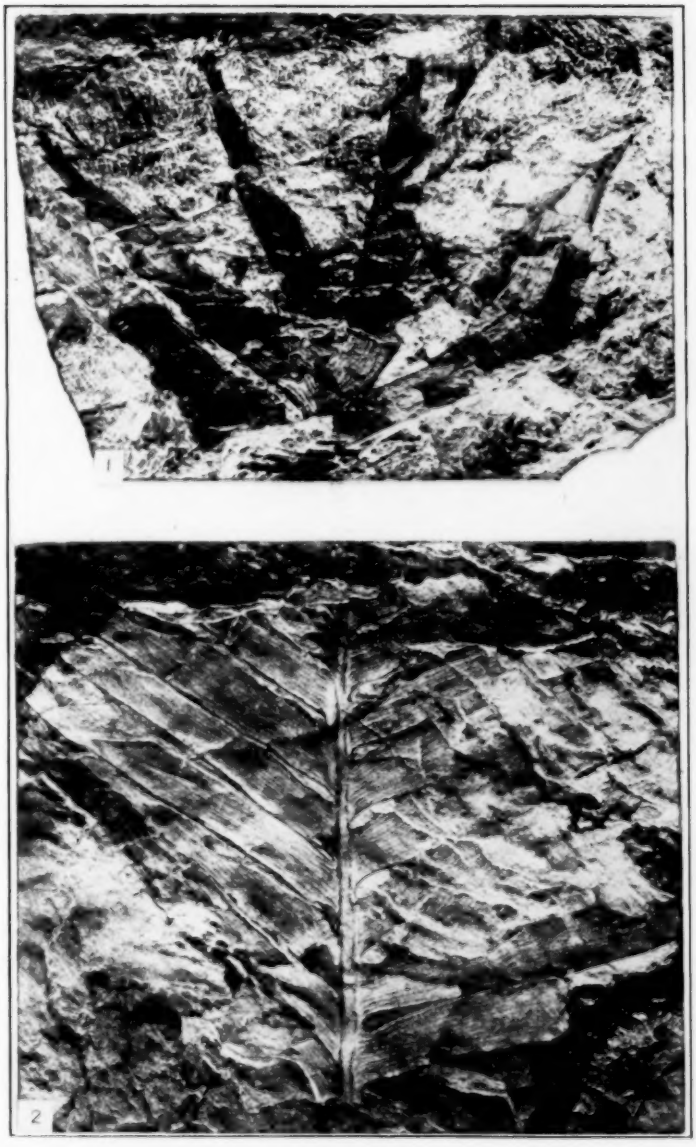

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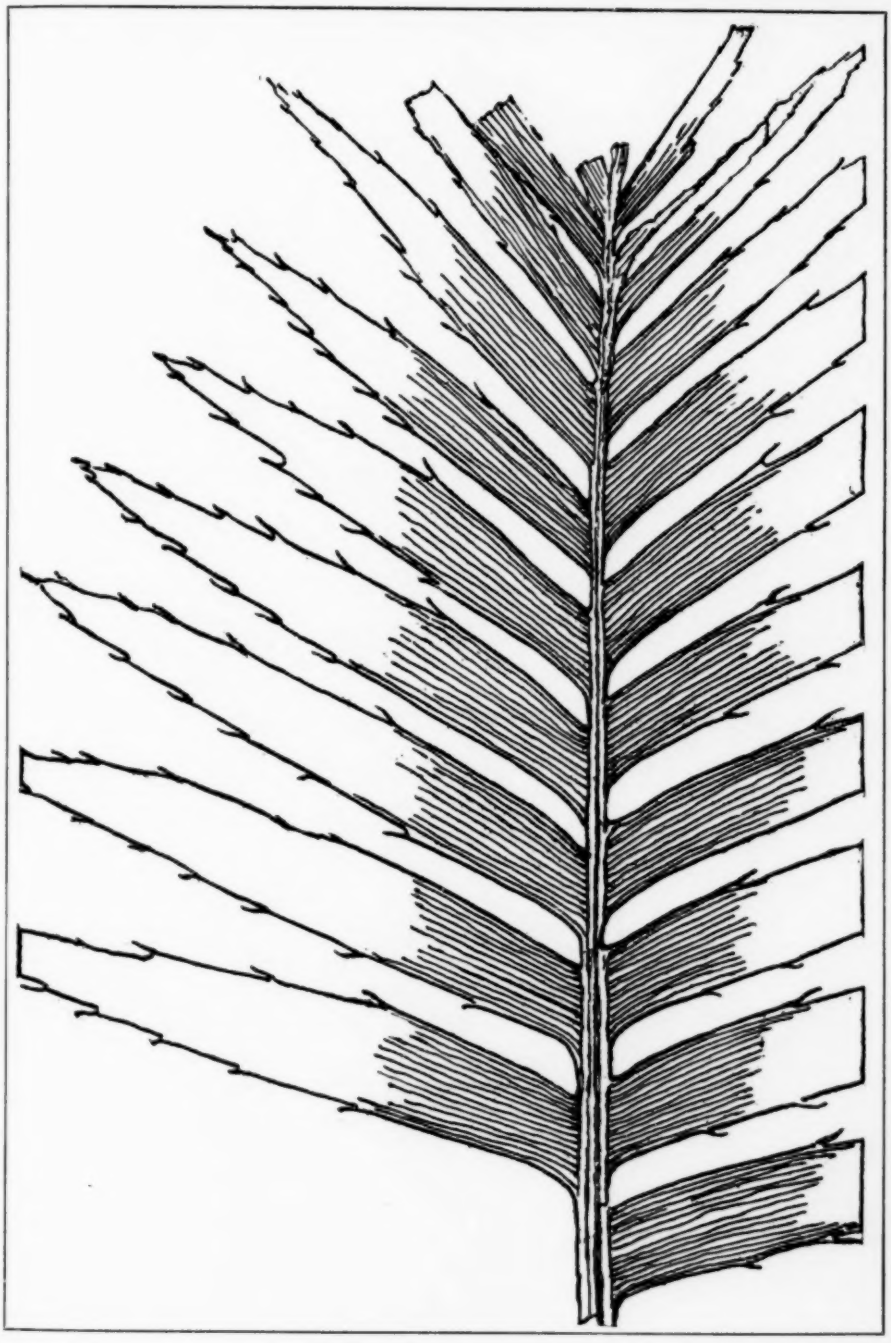

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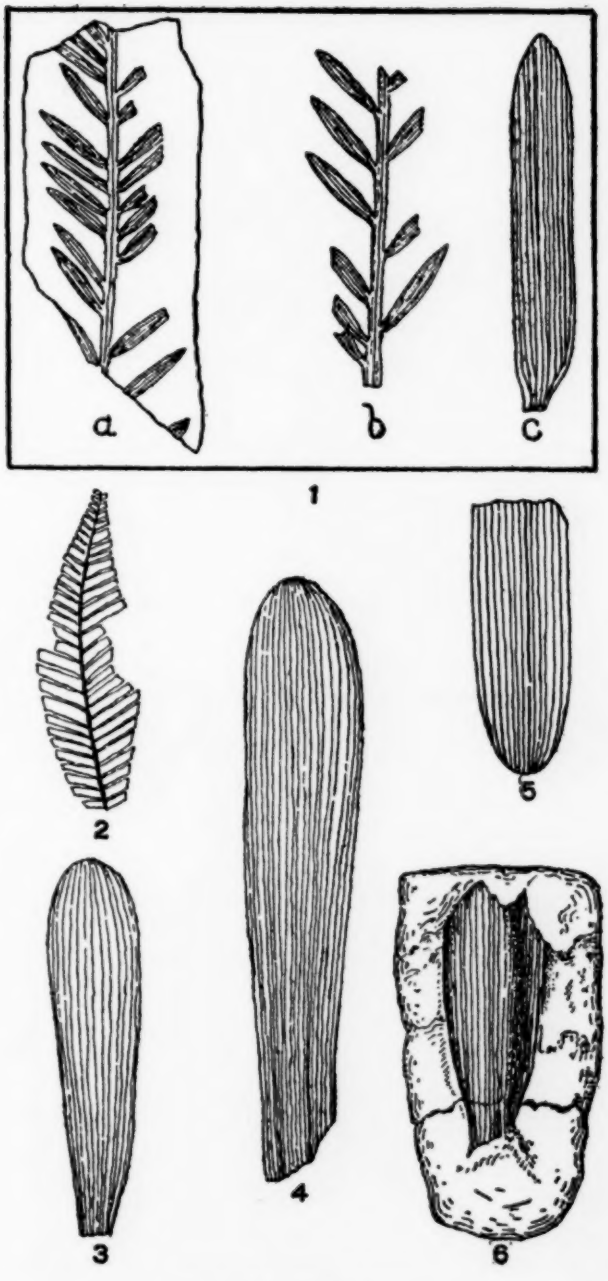

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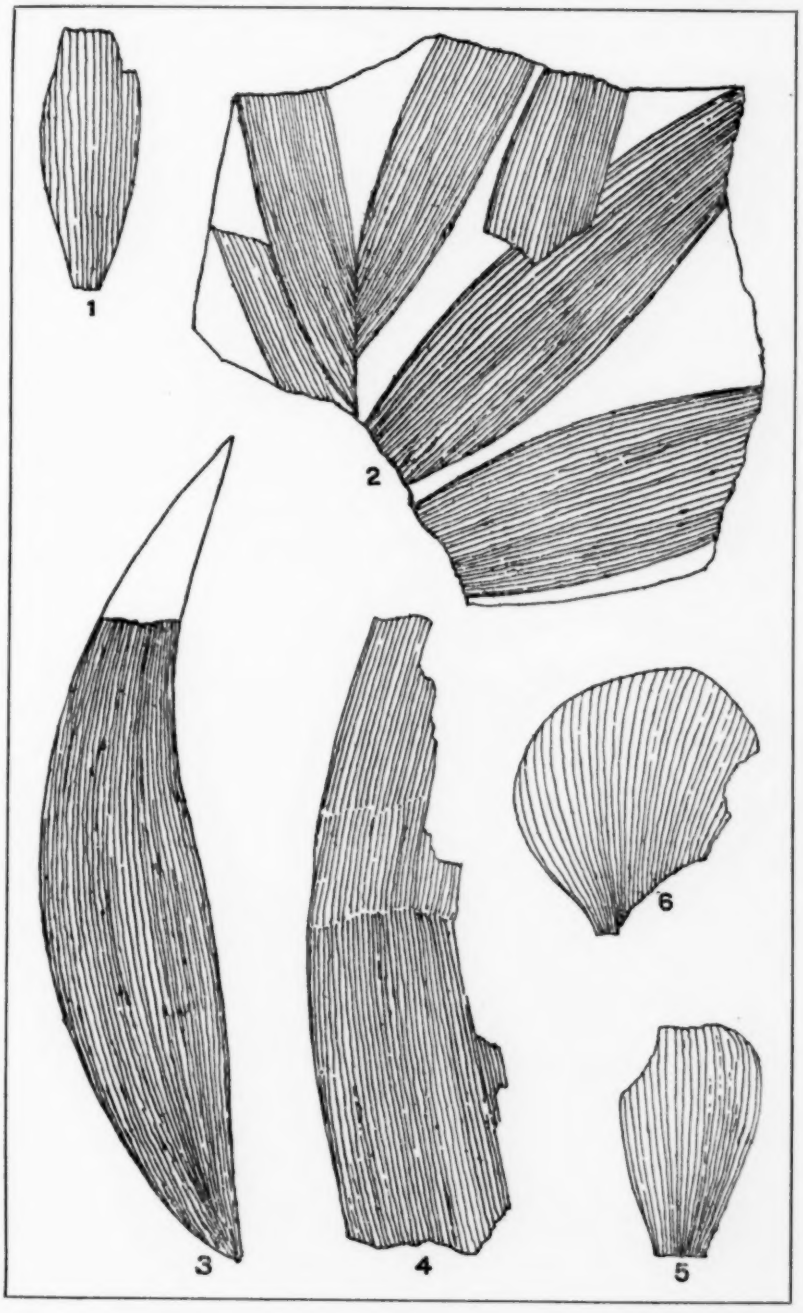

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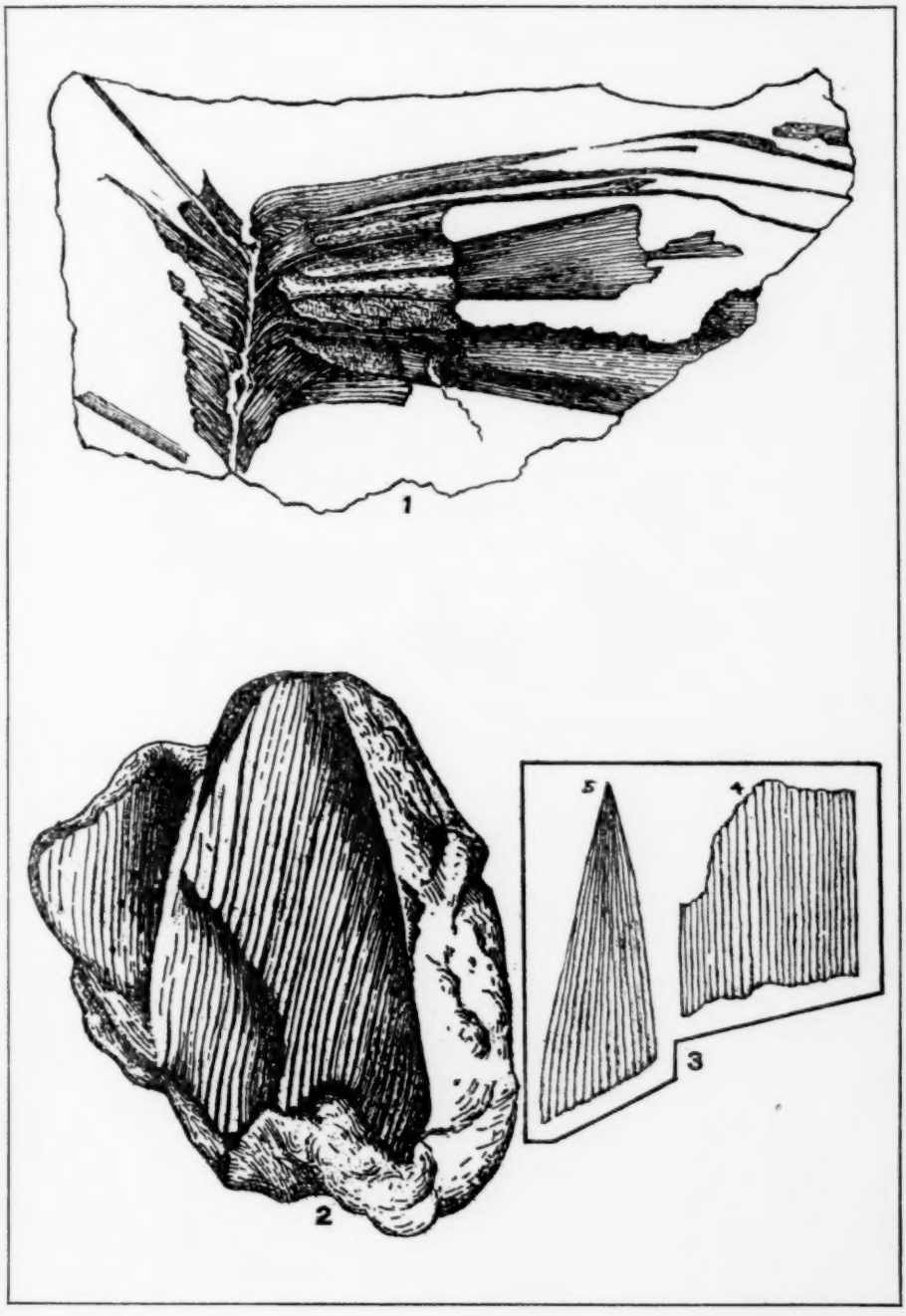

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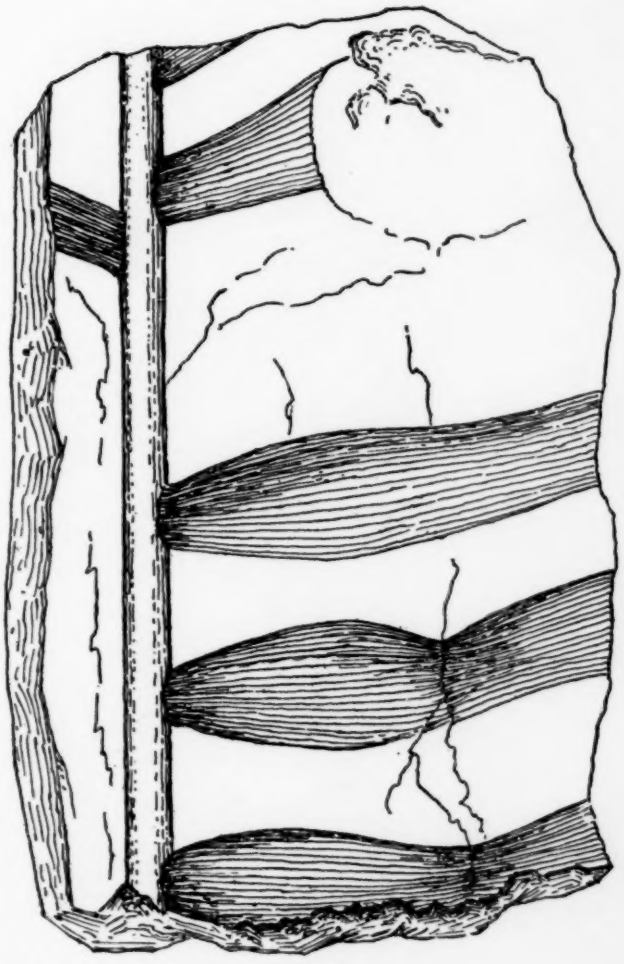

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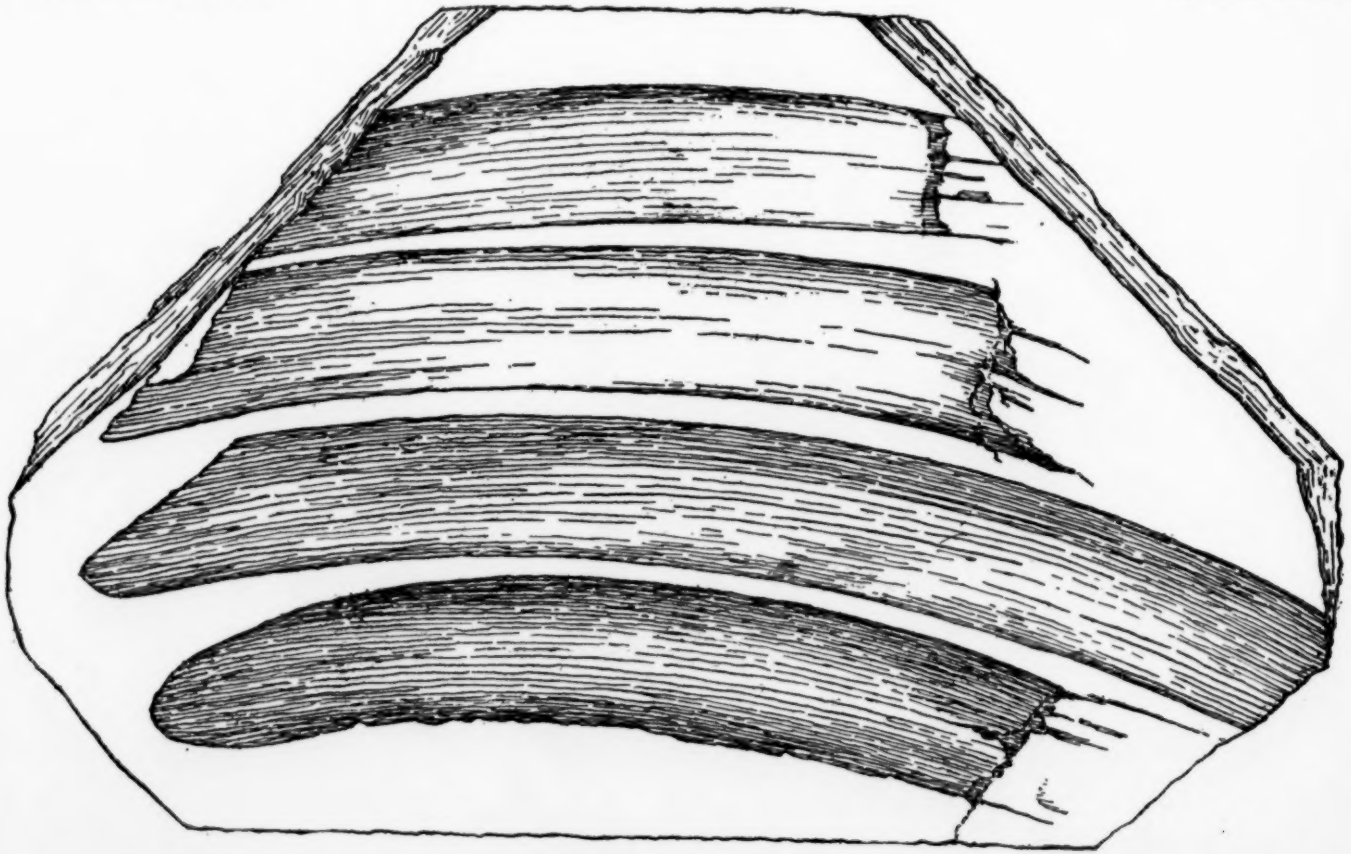

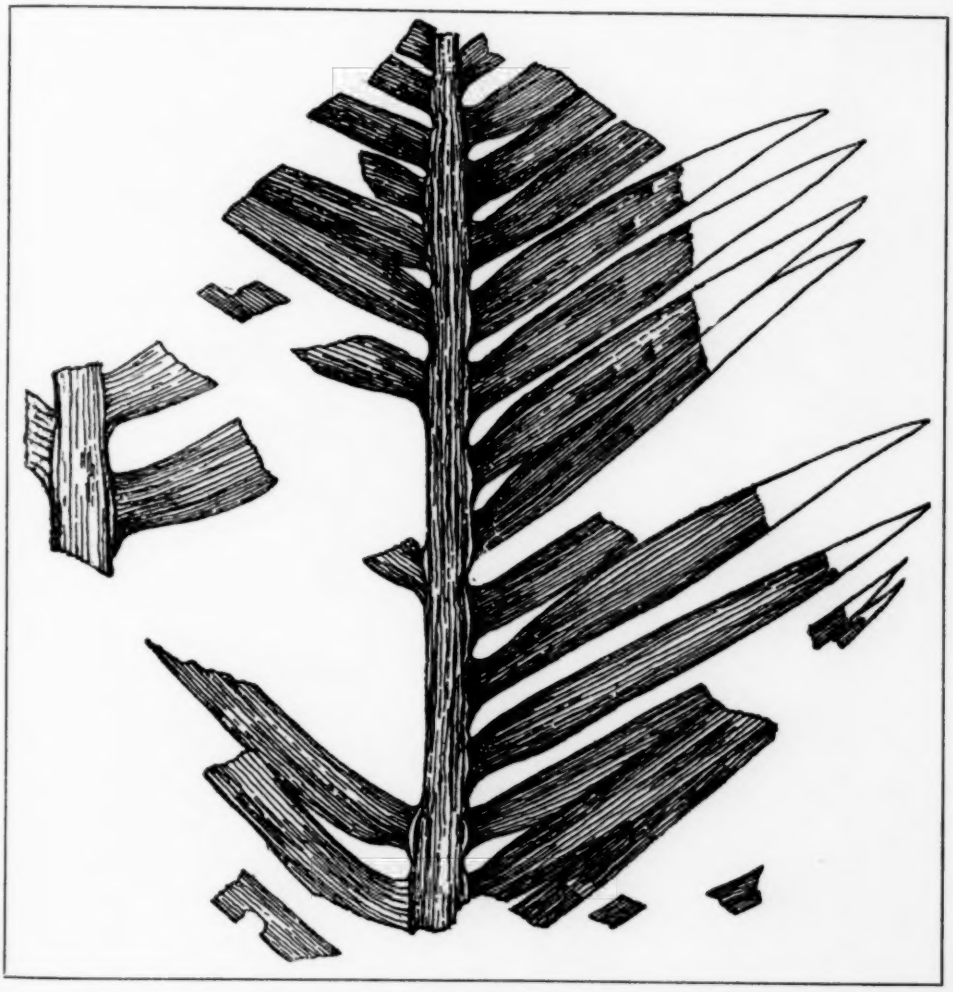

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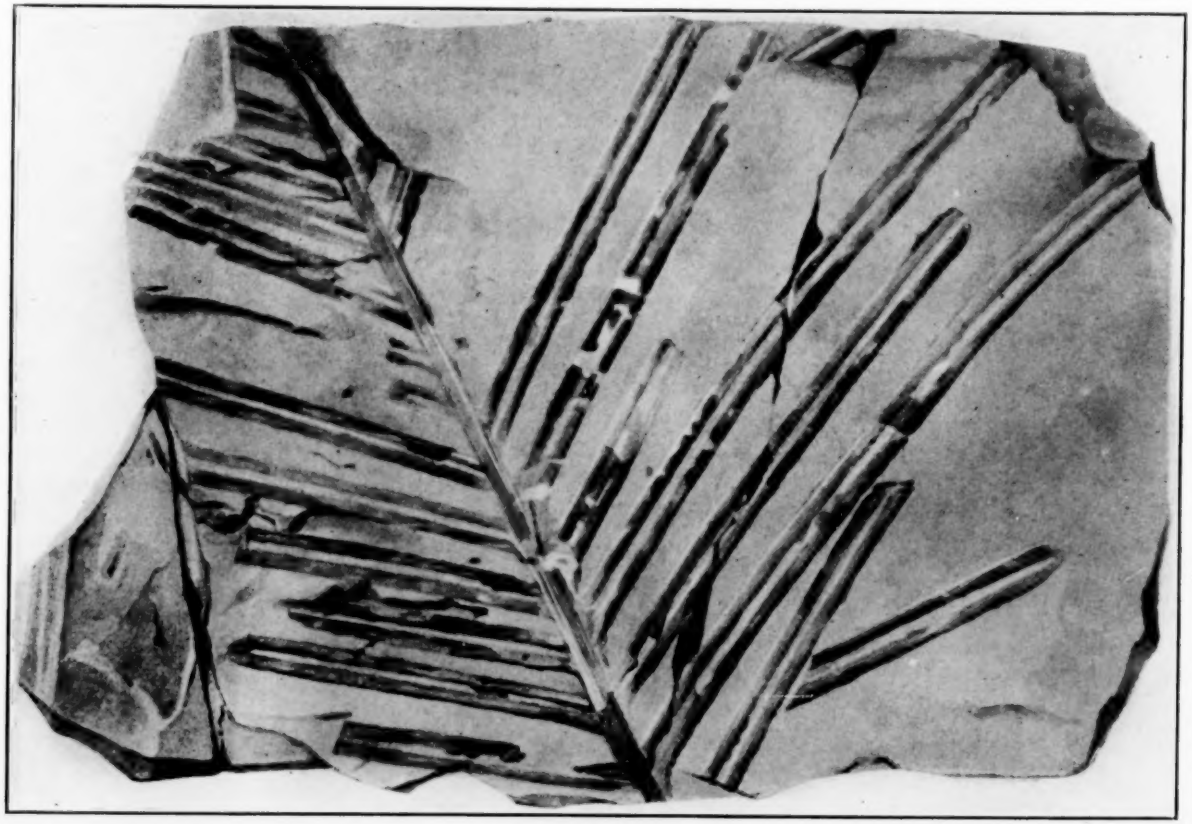

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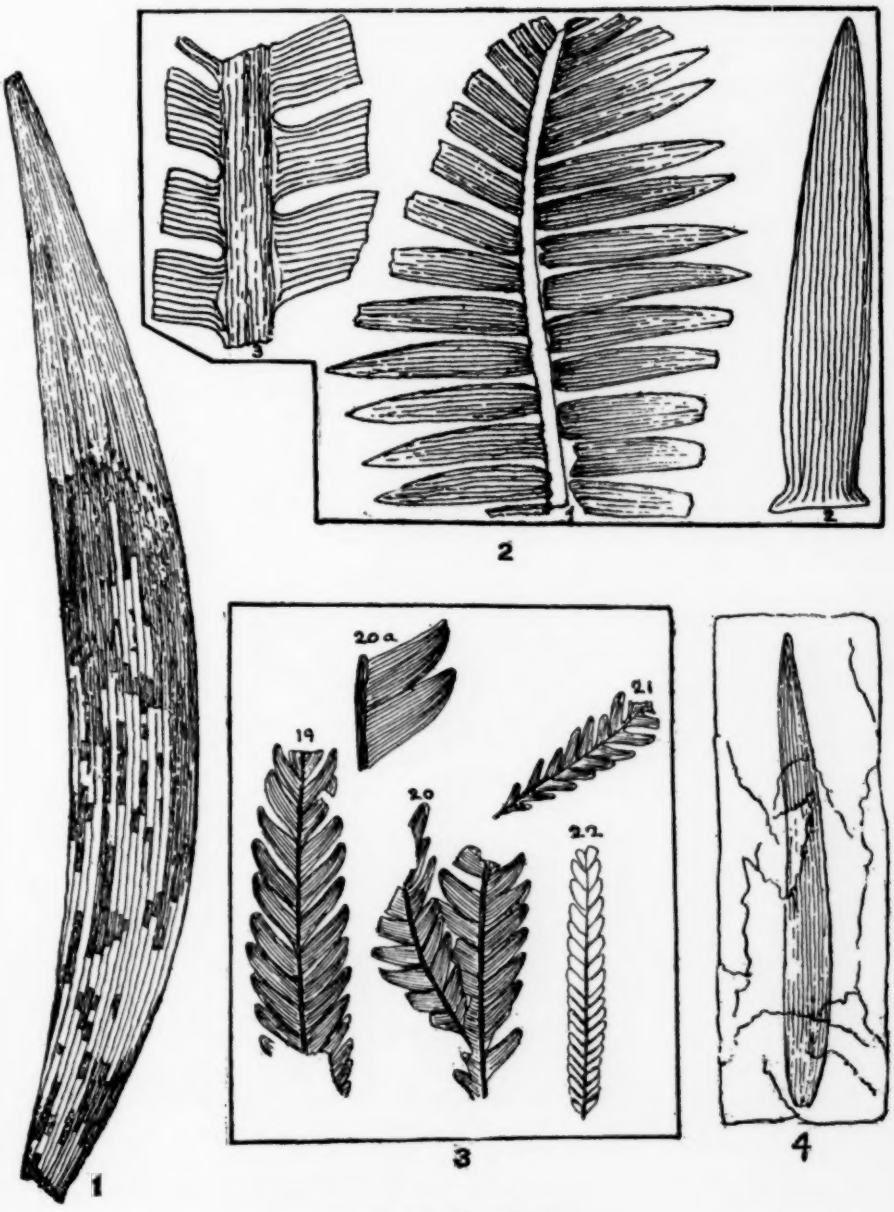

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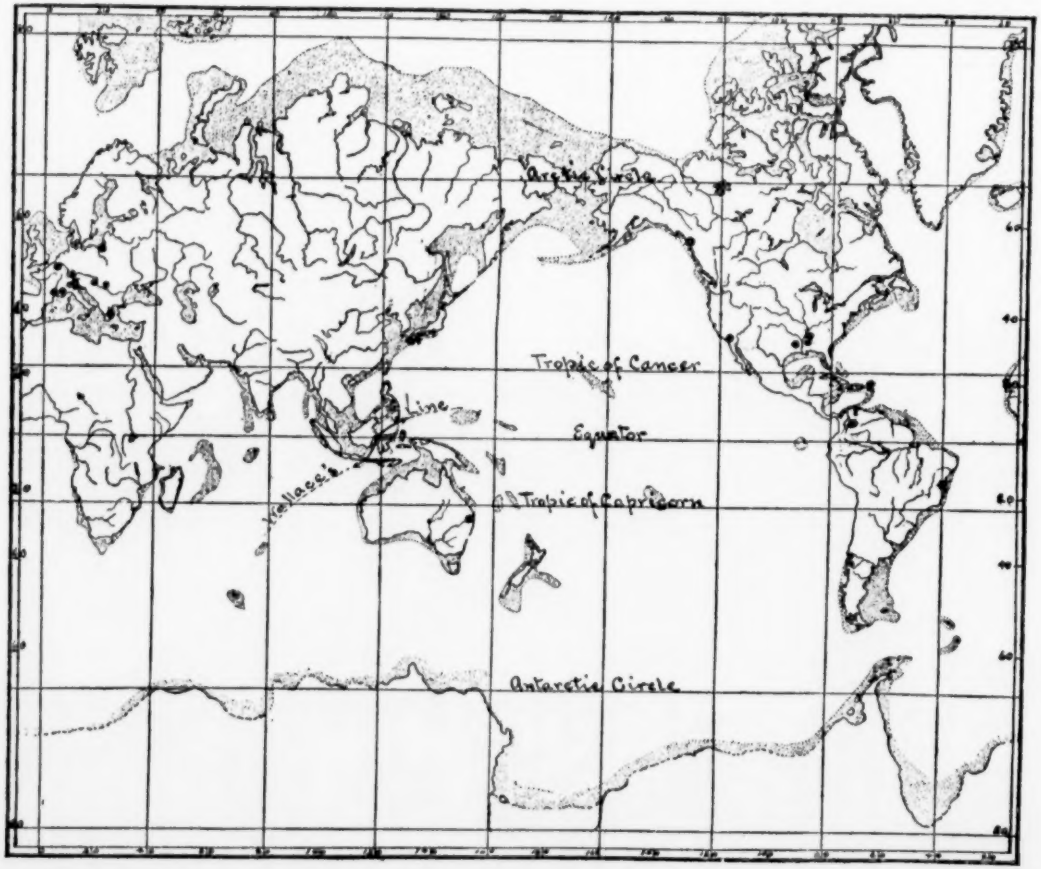

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Inhibiting influence of colloidal starch, inulin, and agar on the stimulation of Aspergillus niger by zinc sulphate

KATHARINE BROWNE STEHLE

(WITH FOUR TEXT FIGURES)

Though a great deal of work has been done upon the growth and stimulation of *Aspergillus niger* in various media, and with various stimulants, little has been done with a colloidal substance present in the solution, either as carbohydrate source, or as an auxiliary substance. The presence of such a substance seems to alter the reaction of the fungus to an inorganic poison such as zinc sulphate.

Kunkel (1913) attempted to determine the toxicity of salts to *Monilia sitophila* in the presence of certain organic substances such as starch, peptone, glucose, fructose, and galactose. He found that the concentrations at which various salts are toxic to *Monilia* depend on the kind of organic substances contained in the media to which the salts are added. The same salt, he said, may be highly toxic in one medium, but quite harmless in another. He did not, however, offer any explanation for this difference in toxicity of the salts in various media.

One of the first experimenters on the effect of metal salts on the growth of *Aspergillus niger* was Raulin (1869). He observed that increased growth resulted when various metallic salts, among them those of zinc, were present in the culture medium. From this he concluded that these elements were all indispensable for normal development of the fungus. Richards (1897) carried on numerous experiments testing the effects of various salts of heavy metals and some organic compounds on the growth of *Aspergillus niger*, *Penicillium glaucum*, and *Botrytis cinerea*. He concluded that these substances were not indispensable to the fungi, but were rather agents which by chemical stimulation caused an acceleration in growth. Steinberg (1919) considered that the greater acidity of the medium due to the presence of the heavy metal ions might possibly explain the increase in growth obtained with zinc salts present. Zinc sulphate is by him, in accordance with the results obtained by Richards, Fred (1911), and other workers, considered as a chemical stimulant causing unusual growth.

The object of the experiments reported in the present paper was to find out whether, when a colloid is present, or when the carbohydrate is presented in starvation quantities, the zinc sulphate has any effect on the dry weight of the yield of mycelium, or whether the amount of zinc sulphate which can be tolerated by the fungus is in any way altered.

METHODS

The methods used in these experiments were similar to those of others who have worked on the stimulation of *Aspergillus*. Pyrex glass-ware was used. Steinberg (1918) concluded that, since cultures of *Aspergillus* grown in Pyrex glass were lower in weight than those grown in other kinds of glass, the Pyrex glass does not add any stimulant, such as zinc, to the solution. The glass-ware that came in contact with the culture solutions was soaked at least overnight in a cleaning solution (sodium dichromate and sulphuric acid), then rinsed thoroughly in tap water, then twice in distilled, and once in double-distilled water. The materials used for the culture solutions were the 'Reagent' grade of Merck's chemicals, with the exception of the starch, inulin, sugar, and agar. Of these last mentioned, the starch and inulin were the 'improved Pfansteil' of the Special Chemicals Company, the sugar was the crystalline form of Kahlbahn's, and the agar 'Difco' standardized. Double-distilled water was used in all cases, the second distillation being through a quartz tube.

The culture medium used was that known as the Pfeffer's solution, containing .5 per cent potassium phosphate (monobasic), .25 per cent magnesium sulphate ($MgSO_4$ 7 H_2O), and 1 per cent ammonium nitrate. Iron is needed only in very small quantities; no iron salt was specially added, as it was assumed that sufficient amounts of iron salts might be present as an impurity in the three main salts. Various concentrations of carbohydrate were used. In making up the culture medium, the solutions were sterilized in an Arnold steam sterilizer, the sugar solutions for thirty minutes, the solutions containing agar for an hour. This was necessary in order to dissolve the agar. The starch and inulin solutions were made by mixing a small amount with water, and then adding this slowly to the culture medium which was boiling. Fifty cubic centimeters of medium were placed in each culture flask (150 cc. Erlenmeyer).

Zinc sulphate was added, for the stimulation series, in fractions of a volume-normal solution. For each series double-distilled water was added to the control, and to each of the flasks containing lower concentrations of the stimulant, so that there would be an equal volume for every flask. In the cases where it would have been necessary to add 2 cc. or more, the nutrient solution was made up double strength, and 25 cc. were put in each flask. The remaining 25 cc. were added by using the desired proportions of normal zinc sulphate and double-distilled water.

The stock cultures of *Aspergillus niger* were grown on white bread, sterilized for half an hour in the Arnold sterilizer before being inoculated. For inoculating each culture flask two loopfuls of spores were taken from the stock culture with a platinum needle.

The cultures were incubated at a temperature of 34.5°C. for four days. Notes were taken at the end of that time on the macroscopic appearance of the felts, fruiting, folds in the felts, yellow coloration, etc. The mycelial felts were then placed on weighed filter-papers, washed with distilled water, and dried in a hot-air oven at 60° for three days. If the dried felts and papers could not be weighed immediately, they were removed to a calcium chloride dessicator. In the cases where the felts were not complete, and therefore could not be removed from the liquid in one piece, the contents of the culture flask were put through the filter paper. In the case of the solutions containing agar, it was found that none of the agar went through the paper, and therefore the weights were correspondingly higher. In all the cultures that were reaped with the medium, 125 mg., the amount of agar allowed for each culture flask when the series were set up, was subtracted from the weight to allow for this. It was also found, by weighing five filter papers before and after they had been in the drying-oven for three days, that the papers lost an average of 15 mg., due to water content, which amount was added to all the averages.

EXPERIMENTAL RESULTS

It was first noticed that, using a 3 per cent starch solution, the macroscopic appearance of the felts was hardly different for those grown with, and those grown without zinc in the medium. Other observers have found, using sugar alone as the carbohydrate, that the external appearance of the fungus mycelium is greatly altered by the presence of small amounts of inorganic stimulants, such as zinc sulphate. Richards (1910) has described this appearance as follows:

> Fungi commonly cease to form conidia under stimulation; and mycelial felts are buckled and knotted instead of being flat and even and their consistency is different, being tough and leathery instead of somewhat tenuous in texture as in the normal growth.

In the case of cultures grown with 3 per cent starch as the carbohydrate, (table 2) however, the felts, on an average, fruited almost as well, at least, as the control up through a concentration of .009 N zinc sulphate. The abundance of spore production then decreased with higher concentrations, down to none in most cases at .031 N and .036 N. Richards (1897) reported that, using 5 per cent sugar as carbohydrate source, with a concentration of .0001 N zinc sulphate present, conidia ripened two days later than in the zinc-free solutions, and with .001 N zinc sulphate, spore formation did not occur at all. In the starch series, all but the control cultures showed some folds in the felts (table 3), but these were almost negligible compared with the folds of a stimulated felt grown in a nutrient

solution containing sugar. The consistency, too, of the mycelium was different, the starch felts being soft and pliable, as compared with the tough leathery felts of sugar solutions. The yellow coloration of nutrient solution found by Richards (1897) to occur usually in the presence of zinc was not constant either for stimulated or control felts.

A comparison may be made of the results obtained from series with 3 per cent starch (table 14) and 3 per cent sugar (table 12) in the nutrient solution, and with various concentrations of zinc sulphate present. The dry weight of fungus obtained in each case in the control solution was practically the same. It may be then that sugar and starch were of like

TABLE 1

Effect of different carbohydrates in the culture medium on the growth stimulation of Aspergillus niger by zinc sulphate

CARBOHYDRATE	HIGH-POINT	INCREASE OVER CONTROL	.0005 VS. CONTROL
Sugar .25%	.0001 N	6.5%	Decrease 23.4%
Sugar .25% + Agar	Control	—	" 24.7%
Sugar .5 %	.0003 N	12.9%	" 2.5%
Sugar .5 % + Agar	.002 N	23.5%	" 7.3%
Sugar 1 %	.004 N	2.3%	" 10.5%
Sugar 1 % + Agar	Control	—	" 6.8%
Sugar 1.5 %	.002 N	73.2%	Increase 61.1%
Sugar 2 %	.0003 N	58.2%	" 52.2%
Sugar 3 %	.0003 N	47.4%	" 40.7%
Sugar 3 % + Agar	.027 N	24.1%	" 16.4%
Starch 3 %	.001 N	14.3%	" 5.6%
Inulin 1 %	.018 N	15.5%	" 9.0%

value as an energy source for *Aspergillus*, and so the results of using these two nutrients may be compared with fairness. If the curve of dry weight plotted against concentration of zinc sulphate (fig. 1) be compared for these two, it will be seen that both rise with increased amounts of zinc sulphate, with the high point for 3 per cent sugar at .0003 N, and for 3 per cent starch at .001 N. In the case of the sugar series, however, the percentage increase of the weight at .0003 N over that with no stimulant present, is 47.4 per cent; in the case of the starch, at the high point it is only 14.3 per cent. In both cases there is a slight drop in yield at .002 N; but for higher concentrations the curves differ markedly in slope. The sugar curve drops off definitely and gradually to a point between .04 N and .045 N, where there is no longer any growth; the starch curve, on the

other hand, is almost a straight line over a wide range of zinc concentrations, exhibiting no tendency to fall until a concentration of .031 N is reached. At .036 N the percentage decrease of the starch culture below the weight of the control is only 30.7 per cent as against 71.7 per cent for the

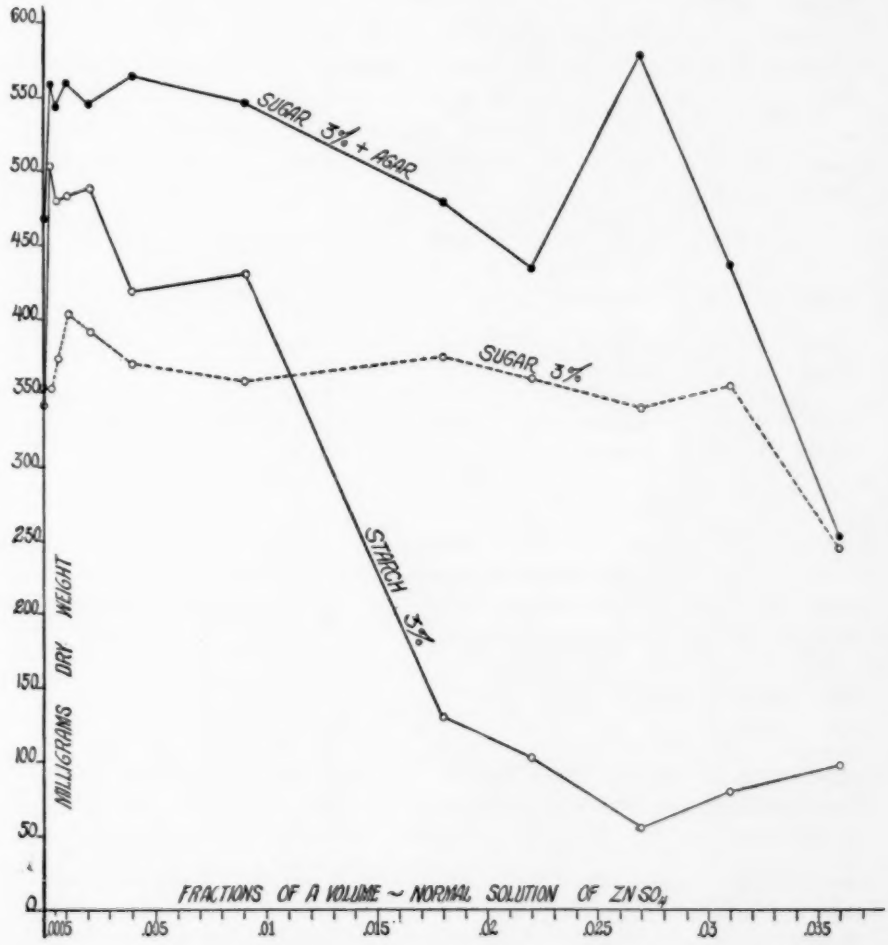

Fig. 1. Growth of *Aspergillus niger*, as influenced by $ZnSO_4$, using 3% sugar, 3% starch, and 3% sugar with agar as carbohydrate.

sugar culture. These results show very clearly that the presence of colloidal starch in the culture solution had a pronounced effect in lowering the toxicity of the zinc sulphate, but no attempt was made to find the lethal concentration of zinc sulphate under these conditions. The increase over the control weight at the high point was only 14.3 per cent when

TABLE 2

Macroscopic appearance of felts: fruiting

CARBOHYDRATE	GOOD FRUIT	SOME FRUIT	LITTLE FRUIT	NO FRUIT
Sugar .25%	Control—.002			
Sugar .25% + Agar	Control—.036			
Sugar .5 %	Control—.002			
Sugar .5 % + Agar	Control—.022	.027	.031	.036
Sugar 1 %	Control	.0001—.002	.004	.009 & higher
Sugar 1 % + Agar	Control—.099	.018, .022, .027		.031 & higher
Sugar 1.5 %	Control	.0003	.0005—.002	
Sugar 2 %	Control		.0005, .0003, .002	
Sugar 3 %	Control		.0001	.0003 & higher
Sugar 3 % + Agar	Control		.0001—.004	.009 & higher
Starch 3 %	Control—.009	.018—.027,	less and less	.031, .036
Inulin 1 %	Control—.018		.036	

TABLE 3

Macroscopic appearance of felts: stimulation

CARBOHYDRATE	FELTS FOLDED BUT FRUITING	'REAL STIMULATION'	YELLOW
Sugar .25%	None	None	None
Sugar .25% + Agar	None	None	1—.0001
Sugar .5 %	.0003, 1—.0005, .002	None	None
Sugar .5 % + Agar	5—.0001, 3—.0003, 4—.0005, .001, .002, .027, .031	None	None
Sugar 1 %	All but control up to .009	.009 to end	One control, one or two of rest
Sugar 1 % + Agar	All but control	None	All in some cases
Sugar 1.5 %	All but control	None	All but control
Sugar 2 %	All but control	None	All but control
Sugar 3 %	None	All but control	Varied
Sugar 3 % + Agar	None	All but control	Varied
Starch 3 %	All but control, except .0003 in some cases	None	Varied
Inulin 1 %	.001—.018	None	Varied

starch was present, as compared with the increase of 94.7 per cent found by Richards (1899) using 5 per cent sugar as the carbohydrate.

Inulin in a 1 per cent solution was also used as a carbohydrate source. The effects of the colloid here may be clouded by the effects of a poor nutrient solution, which will be discussed later. Not enough series were conducted to make the results with inulin sufficiently reliable for precise conclusions. The macroscopic appearance of the felts (tables 2 and 3) was even more striking here than in the starch series; the cultures that contained .018 N or less of zinc sulphate produced as abundant conidia as did the control. At the next higher concentration tried, .036 N, there was still a slight amount of spore production. In some cases the .0005 N cul-

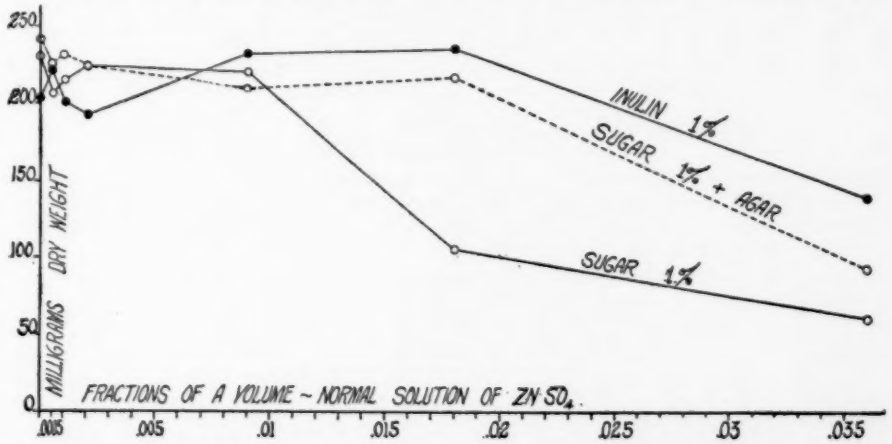

Fig. 2. Growth of *Aspergillus niger* as influenced by $ZnSO_4$, using 1% inulin, 1% sugar, and 1% sugar with agar as carbohydrate.

tures showed no folds; in others all the cultures containing zinc were slightly convoluted. Again, the yellow color was not a constant characteristic of either stimulated or control felts. The high point (table 15) for cultures containing this concentration of inulin was obtained with .018 N zinc sulphate; this point represents an average increase in weight over the control of 15.5 per cent (table 1). At .0005 N the increase over the control was 9.0 per cent. No attempt was made to determine the lethal point. At .036 N, the highest concentration of zinc sulphate used, there was a decrease below the weight of the control felt of only 31.2 per cent. By comparing the graphs of figure 2 showing dry weight plotted against zinc sulphate concentration for culture media containing 1 per cent inulin, 1 per cent sugar, and 1 per cent sugar with agar, it will be seen that the inulin curve follows more closely that of the sugar and agar, when the higher zinc sulphate concentrations are considered.

TABLES 4-14

Dry-weights of felts of Aspergillus niger, with zinc sulphate added to the culture medium in fractions of a Normal solution

In the following tables, to each of the final averages is added 15 mg. to allow for weight lost from the filter papers in the drying oven.

Weights marked * are those where the felts were not complete, and the weight of culture medium is included. From each of these, 125 mg. was subtracted, to allow for the weight of dried agar.

Cultures were incubated 4 days, at a temperature of 34.5°C. Weights are in milligrams.

TABLE 4

Sugar .25 per cent

−Zn	.0001	.0003	.0005	.001	.002
91			75		
93	99	64	59	95	64
107	114	79	82	110	79

TABLE 6

Sugar .5 per cent

−Zn	.0001	.0003	.0005	.001	.002
105			112		
150		146	136		106
142.5		161.0	139.0		121.0*

TABLE 5

Sugar .25 per cent, Agar .25 per cent

−Zn	.0001	.0003	.0005	.001	.002	.004	.009	.018	.022	.027	.031	.036
91	90	99	94	86	74							
106	66	93	79	115	91							
156	114	77	68	91	59	89	69	109	58	87	141	96
						88	84	98	85	90	113	66
93											128	82
											147	173
											143	147
126.5	105.0	104.7	95.3	112.3	89.7	103.5	91.5	118.5	86.5	103.5	94.4	77.8

TABLE 7

Sugar .5 per cent, Agar .25 per cent

−Zn	.0001	.0003	.0005	.001	.002	.004	.009	.018	.022	.027	.031	.036
147	136	137	131	145	148							
95	157	172	139	136	109							
126						131	131	109	111	129	146	138
						126	101	138	128	111	144	
						125	105	110	112	119	120	
240					261	199	167	187	281	210	119	279*
					141	182	200	143	139	112	336*	267*
					285							190*
												242*
142	98	141	136	147								
	148	163	164	121								
	146	114	120	141								
165.	152.	160.4	153.	153.	203.8	167.6	155.8	152.4	169.2	151.2	163.	138.2*

*15 mg. added to weight to allow for loss of weight from filter paper.

TABLE 8
Sugar 1.5 per cent

−Zn	.0001	.0003	.0005	.001	.002
183		287	304		328
198		302	319		343[a]

TABLE 9
Sugar 2 per cent

−Zn	.0001	.0003	.0005	.001	.002
234		379	364		378
249		394	379		393[a]

TABLE 10
Sugar 1 per cent

−Zn	.0001	.0003	.0005	.001	.002	.004	.009	.018	.022	.027	.031	.036	.04	.045	.05	.1	.2
171		204	225		227												
202	179			215	207	210	214	39	68	24	15	46					
216	154	163	143	161	170	207	200	120	85	77							
	150	193	178	166		244	207	100	98	86							
311	179	212	190	225	194	225	203	66	107	101	110	—					
204	197	166	214	228	193	210	199	121	123	94	50	34					
											41	37					
											166	56					
213															—	—	—
244													206	—	—		
186											36	96	—				
213											124	40	—				
180													—				
													—				
229.0	186.8	204.6	205.0	214.0	223.2	234.2	219.6	104.2	111.2	91.4	92.4	59.1	56.2	—	—	—	—[a]

TABLE 11
Sugar 1 per cent, Agar. 25 per cent

−Zn	.0001	.0003	.0005	.001	.002	.004	.009	.018	.022	.027	.031	.036	.04	.045	.05	.1	.2
197	194	202	194	209	186												
219	247	201	256	223	214												
218									204	230	217	128*					
194	197	186	204	238	239	233	210	207	144	164*	161*	156*					
						192	201	211	225	177*	162*	200*					
225										206	212	215*					
										217	191	213*					
194	189					201	208	211	189								
						173	175	203	205								
						189	182	181	217								
178			168			202											
229	209	234	224	211	202												
		222		200	201												
326															199*	313*	—
226													172*	160*	257*	167*	—
231												252	189*	199*	243*	—	
288											180*	133*	143*	125*	118*		
207													297*	179*	196*	—	
													202*	234*		—	
240.5	222.2	224.0	224.2	231.2	223.4	213.3	210.2	217.6	212.3	163.8	139.7	93.1	90.6	69.4	92.6	0	—[a]

[a] 15 mg. added to averages to allow for loss of weight from filter papers.

The lack of stimulation found in the starch and inulin cultures was at first attributed wholly to an occluding effect that the disperse phase of the colloidal solution might have upon the poison. The same lack of stimulation, however, was found when *Aspergillus* was grown in media contain-

TABLE 12
Sugar 3 per cent

−Zn	.0001	.0003	.0005	.001	.002	.004	.009	.018	.022	.027	.031	.036	.04	.045	.05	.1	.2
271		494	520		542												
271		471	439	458	483	576	475	88	148			120					
284	391	432	373	429	488	429	218	58	30								
220																	
327																	
262	509	532	475	460	377	499	511	214	73	46	53	13					
	474			468		382	444	155	141								
	497																
477															—	—	—
482													311	—	—		
339											194	137	—				
293											25	91	82				
422	483	508	516	517	476	131	433	61	46	45							
										5							
										40							
										65							
263											23	47	26				
											28		14				
340.9	485.8	502.4	479.6	481.4	488.2	418.4	431.2	130.2	102.6	55.2	79.6	96.6	101.6	—	—	—	—[a]

TABLE 13
Sugar 3 per cent, Agar .25 per cent

−Zn	0001.	0003.	0005	.001	.002	.004	.009	.018	.022	.027	.031	.036	.04	.045	.05	.1	.2
400	457	534	518	514	588	489	489	563	550	542	250*	379					
431																	
454																	
467	566	586	569	581	492	528	557	619	525	525	505	416*					
285			500			492											
345										646	528	356*					
										503	580	375*					
										604	541	540					
533															177*	200*	—
797													307*	270*	344*	437*	—
496												227*	251*	194*	418*	—	
393											256	141*	178*	146*	313*		
464	496	517	518	529	525	641	558	480	225*								
	507	509	537	541	525	598	534	526*	561*								
	501	571		559	522		518	390*	494								
355													145*	128*	214*	—	
													316*	151*		—	
466.7	520.4	558.4	543.4	559.8	545.4	564.6	546.2	480.6	436.0	579.0	437.5	255.6	129.4	67.8	183.2	17.4	—[a]

[a] 15 mg. added to average to allow for loss of weight from filter papers.

ing very low percentages of sugar (between .25 per cent and 1 per cent). It cannot be entirely due to starvation, however; for in the case of the 3 per cent starch control and the 3 per cent sugar control, the dry weights were practically the same, yet there was a decided difference between the

appearance of the mycelia grown in solutions containing starch and zinc and the appearance of the mycelia of similar solutions with sugar as the carbohydrate. In order to separate better this starvation effect from the colloidal effect, sugar was used as the carbohydrate in varying concentrations and .25 per cent agar was added to the solutions in which the effect

TABLE 14

Starch 3 per cent

−Zn	.0003	.0005	.001	.002	.004	.009	.018	.022	.027	.031	.036
315	337	318	336	338	315	326	338				278
388		299	413	360	364	342	294	344	286		171
367		311	465	349	373	358	362	327	270		256
377		365	377	368	347		400				198
366		412	369	352	364		352				259
388		481	393	384	359		436				70
339		396	362	479	355		408				182
310		324					342	315	326	276	346
282		305					347	341	344	335	140
345		386					386	373	281	337	287
305		376					343	354	358	281	249
304		324					325	357	375	417	285
302		347					331	344	356	371	301
352.5	352.0	372.2	402.9	390.7	368.9	357.0	373.8	359.4	339.5	354.5	244.4[a]

[a] 15 mg. added to weight to allow for loss of weight from filter paper.

No correction was made for any starch that may have adhered to the filter paper when felts were reaped.

TABLE 15

Inulin 1 per cent

−Zn	.0005	.001	.002	.009	.018	.036
178						
174	200					83
181	173	184	174	213	194	
208		188	182	222	246	167
201	247					
203.4	221.7	201.0	193.0	232.5	235.0	140.0[a]

[a] 15 mg. added to weight to allow for loss of weight from filter paper.

of a colloid was to be tested. It was found that agar alone would not produce appreciable growth; so it was assumed that the agar was of little or no value as food. In each case where agar was used the control weight was a little higher, but the difference was not very great. The data secured in these tests are presented in tables 4 to 13, and are shown graphically in figure 3.

TABLE 16

Comparison of average of dry weights of mycelium of Aspergillus niger obtained with different carbohydrates in the culture medium; and of the effect on these of the presence of various fractions of a volume-normal solution of zinc sulphate.

CARBOHYDRATE	CONTR.	.0001	.0003	.0005	.001	.002	.004	.009	.018	.022	.027	.031	.036	.04	.045	.05	.1
Sugar .25%	107.0	114.0	79.0	82.0	110.0	79.0											
Sugar .25% + Agar	126.5	105.0	104.7	95.3	112.3	89.7	103.5	91.5	118.5	86.5	103.5	94.4	77.8				
Sugar .5 %	142.5		161.0	139.0		121.0											
Sugar .5 % + Agar	165.0	152.0	160.4	153.0	153.0	203.8	167.6	155.8	152.4	169.2	151.2	163.0	138.2				
Sugar 1 %	229.0	186.8	204.6	205.0	214.0	223.2	234.2	219.6	104.2	111.2	91.4	92.4	59.1	56.2	—	—	—
Sugar 1 % + Agar	240.5	222.2	224.0	224.2	231.2	223.4	213.3	210.2	217.6	212.3	163.8	139.7	93.1	90.6	69.4	92.6	0
Sugar 1.5 %	198.0		302.0	319.0		343.0											
Sugar 2 %	249.0		394.0	379.0		393.0											
Sugar 3 %	340.9	485.8	502.4	479.6	481.4	488.2	418.4	431.2	130.2	102.6	55.2	79.6	96.6	101.6	—	—	—
Sugar 3 % + Agar	466.7	520.4	558.4	543.4	559.8	545.4	564.6	546.2	480.6	436.0	579.0	437.5	255.6	129.4	467.8	183.2	17.4
Starch 3 %	352.5		352.0	372.2	402.9	390.7	368.9	357.0	373.8	359.4	339.5	354.5	244.4				
Inulin 1 %	203.4			221.7	201.0	193.0		232.5	235.0				140.0				

There seems to be a critical concentration of sugar which lies somewhere between 1 and 1.5 per cent. With sugar concentrations below this, no stimulation of growth was secured with zinc sulphate, either in the absence or presence of agar. With sugar concentrations above this critical concentration, pronounced stimulation of growth was obtained with zinc

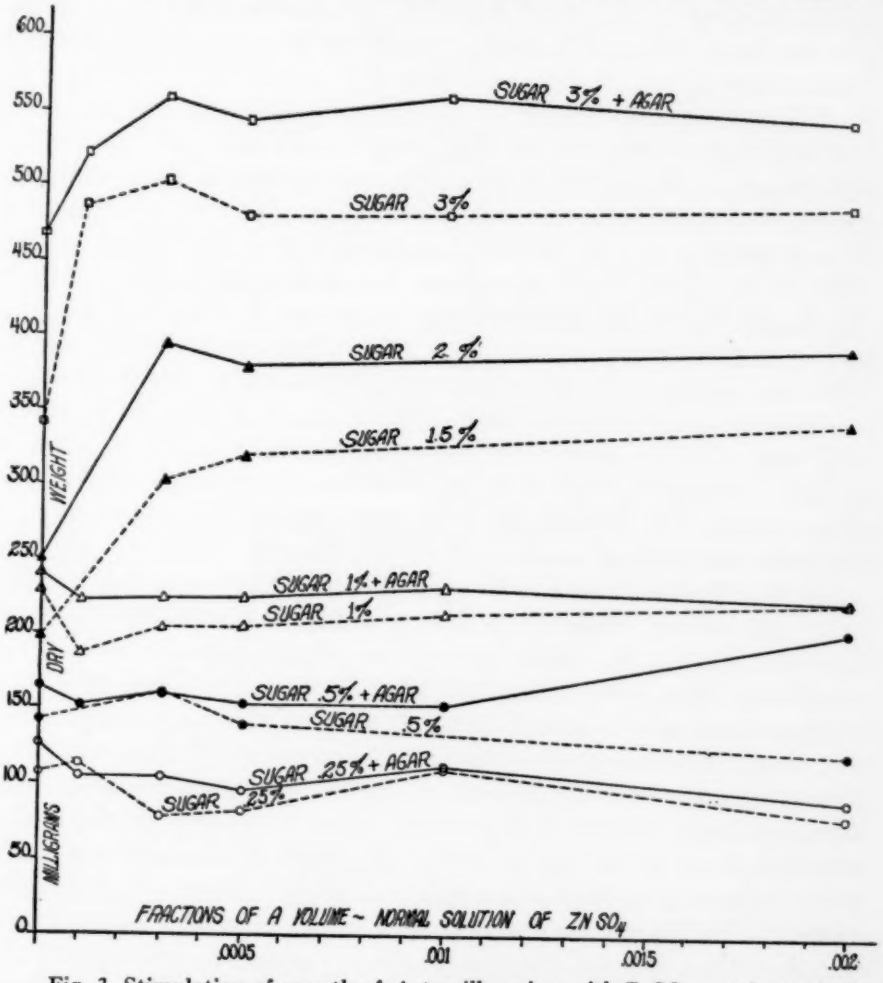

Fig. 3. Stimulation of growth of *Aspergillus niger* with $ZnSO_4$: nutrient solution with various concentrations of sugar, with and without agar.

sulphate in the absence of agar; in the presence of agar, some stimulation occurred, but it was much less pronounced. Thus a complete or partial lack of response to chemical stimulation may occur when the fungus is growing on a poor food supply and may be due to its lowered rates of metabolic processes, or it may occur as the result of the presence of a colloid when the fungus has a favorable food supply.

When a very low percentage of sugar was used, .25 per cent, the mycelial felts were very thin. They were smooth and produced an abundance of spores through concentrations of .002 N zinc sulphate, the highest concentration tested (tables 2 and 3). In no case was there any coloration of medium or felt. With a .0001 N zinc sulphate solution (table 4) there was a slight increase in dry weight of crop, but this amounted to only 6.5 per cent. At .0005 N the weight showed a decrease of 23.4 per cent below that of the control.

Using this same concentration of sugar with agar present, the growths looked practically the same as without agar in the medium. Even at concentrations of .036 N zinc sulphate the fruiting was as dense as in the control, and the felts were smooth. In this series, zinc sulphate seemed to have no effect as a stimulant, the control yield being the highest of any (table 5). No effect, either stimulative or poisonous, was evident until at .031 N and .036 N zinc concentrations, where the yields fell off abruptly, indicating the beginning of the toxic effect of the zinc. With as high as .027 N zinc sulphate, the decrease in weight below that of the control was only 18.1 per cent. The decrease at .0005 N was 24.7 per cent. It will be seen, then, that the response of *Aspergillus* to zinc sulphate in nutrient solutions containing .25 per cent sugar was practically the same with and without agar, with the exception of a slight increase with very low zinc concentrations without agar.

With a doubled amount of sugar present (.5 per cent) the control weight was higher (table 6). This is in accordance with the results of Haenseler (1921), who found that, as the sugar concentration was increased, the dry weights of the yield were proportionally higher. His sugar concentrations were expressed in terms of atmospheres of osmotic concentration, and he reported such an increase up to a concentration of eight atmospheres, or about 11 per cent sugar. The increase found here, with .5 per cent sugar, over the weight of the control grown with the lower carbohydrate is one of 33.2 per cent. There is, however, the same lack of response to stimulation with zinc sulphate as in the less concentrated sugar solutions. The highest amount of zinc added was a .002 N solution, but even there the fruiting was as heavy as on the control felt. In some cases, however, the zinc cultures were beginning to show slight folds. No yellow color was found in any case. The 'high-point' of stimulation for .5 per cent sugar came at the .0003 N zinc sulphate concentration, where there was an increase of only 12.9 per cent over the weight of the control, but this increase may not have been significant. The weights dropped off very gradually from there to zinc sulphate concentrations of .002 N, where the weight of the fungus was 15.1 per cent lower than the control.

Where agar was used with the .5 per cent sugar cultures (table 7), much the same thing happened as with the .25 per cent sugar and agar; that is, there resulted a decrease in crop weight, below the control. Here, however, the yield rises again to zinc concentrations of .002 N, where there is an increase of 23.5 per cent over the dry weight of the control. This increase begins with a concentration of .001 N. If significance is to be attached to this rise, it may mean that when the concentration of sugar is rather low stimulation may occur in the presence of agar, but such stimulation appears only when relatively high zinc concentrations are employed; the appearance of the cultures, however, did not suggest stimulation, and there was little difference when agar was present or absent. The fruiting of felts grown in solutions containing as much as .022 N zinc sulphate was as good as the control felts (tables 2 and 3); there was less spore production at .027 N zinc sulphate, very little at .031 N, and none at all at .036 N. Almost all cultures showed some slight evidence of stimulation, though only in the form of small folds in the felts. No yellow was found here either.

The above results for varying sugar concentrations can hardly be taken as very reliable, except in the case of the series containing .5 per cent sugar and agar, for in the others too few cultures were set up for a good average. More experiments on these series should be done before definite conclusions can be drawn.

With 1 per cent sugar, the effect of starvation amounts of carbohydrate were still very pronounced, as seen in the dry weight of mycelium produced and, to a lesser degree, in the appearance of the felts (tables 2 and 3). In no case was the fruiting as good in the zinc cultures as in the zinc-free, though there was good fruiting through concentrations of .002 N, less at .004 N, and no fruit at all at concentrations of .009 N and higher. The control felts were the only smooth ones here. Yellow color of felt or medium was not a constant point of difference between control and zinc cultures. The appearance of the fungous growth in the higher zinc series suggested that of stimulated growth such as has been reported by other workers; there was a lack of fruiting and a folding of the mycelium.

As far as the dry weights at a concentration of 1 per cent sugar are concerned (table 10), with .0001 N zinc sulphate there is a falling off in weight amounting to 18.4 per cent of the control. The reason for this is not understood. The fall in the curve (figure 3) is perhaps due to an abnormally high weight for the control culture. This is suggested by the fact that the control weight for this sugar concentration is higher than for 1.5 per cent sugar, and lies about half way between that for 1.5 per cent and that for 2 per cent sugar. Whatever the cause of the fall may be, it should be noted that a similar decrease in weight at .0001 N zinc sul-

phate occurs where there is 1 per cent sugar with agar in the medium. The dry weight increases gradually from the low point at a concentration of .0001 N to a concentration of .004 N zinc sulphate, where the maximum growth is reached. This can hardly be considered a 'high-point of stimulation', as the increase over the control is only one of 2.3 per cent.

The above results should be compared with those obtained using 1 per cent sugar with agar present. One notices at once a difference in the fruiting (table 2). Through concentrations of .009 N zinc sulphate there was as abundant spore production as in the control. At this same concentration of zinc sulphate (.009 N) without a colloid present no spores were formed at all. With agar present, on the other hand, there was still some spore production, less than the control, to be sure, with .018 N, .022 N, and .027 N concentrations of zinc sulphate; and production of spores did not cease entirely until a strength of .031 N was reached. The presence of a yellow color of felt or medium was again variable. In all cases except the control there was very slight stimulation in the form of small folds in the felts (table 3).

The average weight of the control grown with agar present was very slightly higher than that grown in 1 per cent sugar with no agar (table 11). Except for a slight drop with .0001 N zinc sulphate, the weights at increasing concentrations of zinc sulphate remained practically the same, up through a concentration of .022 N. At no point, however, did the weight of zinc cultures come up to that of the zinc-free cultures.

Using 1.5 per cent sugar in the nutrient solution, the starvation effect on the response of *Aspergillus niger* to the presence of zinc sulphate seemed to stop suddenly. With small amounts of zinc present there was a considerably increased growth which begins to be comparable to that found by Richards and others with a higher sugar concentration. Only a few series were set up with the nutrient containing 1.5 per cent and 2 per cent sugar, and none with agar present at these two concentrations. In each case, also, .002 N was the highest amount of zinc sulphate used. The fruiting in these series was affected by the zinc (table 2); for in the nutrient with 1.5 per cent sugar, there was some fruit where zinc sulphate was present in a concentration of .0003 N, and a very little where .0005 N and .002 N concentrations were present. In the nutrient with 2 per cent sugar there was only a very little fruiting in any zinc cultures. With both 1.5 per cent and 2 per cent sugar all but the control were yellowish and showed signs of folding of the felts (table 3).

With 1.5 per cent sugar the average weight of the felts was considerably higher when zinc was present (table 8), there being an increase of 73.2 per cent over the weight of the control at the high point, .002 N. (This was the highest concentration of zinc sulphate tested.)

The control of the 2 per cent sugar series was about 50 mg. heavier than that of the 1.5 per cent sugar solution (table 9). The weights of the 2 per cent sugar solution followed those of the less concentrated quite closely, except that the high point was at a zinc content of .0003 N. The increase in weight over the control at this point was one of 58.2 per cent.

With 3 per cent sugar the evidences of stimulation, as far as gross appearance is concerned, were even more pronounced (tables 2 and 3). With the exception of the concentrations of .0001 N where there was a very little, the zinc cultures showed no fruiting at all. Here, too, the yellow coloration was not constant. Stimulated felts, like those described by Richards, appeared in all zinc cultures; that is, mycelium showing a lack of fruiting, and tough leathery, much folded vegetative growth.

The dry weights, too, showed this stimulation (table 12), there being an increase of 47.4 per cent over the weight of the control at a .0003 N zinc content, the high point for the series. From here on, the weights decreased gradually with higher amounts of zinc. By comparing the graphs of the dry weight of fungus grown with increasing amounts of zinc in nutrient containing 3 per cent, 2 per cent, and 1.5 per cent sugar solutions (figure 3) it will be seen that they are all very much the same, each higher sugar percentage giving correspondingly higher weights throughout the series.

Where agar is present with the 3 per cent sugar in the nutrient solution, there is some evidence of a reduced response to zinc sulphate (tables 2 and 3). There was a slight amount of fruiting where zinc was present in a .004 N and lower solution. Evidences of stimulation were seen in all the solutions containing zinc, as far as folding of the felt was concerned. In no case, when agar was present, were the felts as tough and leathery in consistency as when only sugar was used in the medium. The presence of yellow here, too, was not constant.

The weights of fungus grown in 3 per cent sugar and agar (table 13) corresponded quite closely to those of the 3 per cent sugar solutions alone, when lower concentrations of zinc sulphate were used. The control weight was considerably higher than when 3 per cent sugar alone was supplied the fungus, but the reaction to zinc was not so pronounced with agar present in the solution. It may be, then, that the control was somewhat stimulated to begin with; but this hardly seems probable, for the macroscopic appearance of the controls in all cases was of a smooth, densely fruiting mycelium. The maximum weight for this series was obtained with a zinc concentration of .027 N, where the increase over the control was 24.1 per cent. At .0003 N, the high point of stimulation for 3 per cent sugar alone, there was, with agar present, an increase of only 19.6 per cent in dry weight, as compared with an increase of 47.4 per cent for sugar alone.

In addition to the inhibiting effect of colloids on the stimulating action of zinc sulphate, it was observed that when a colloid was present in the nutrient solution much greater quantities of the poison could be tolerated by *Aspergillus* than when no colloid was present. Special attention was paid to this, and to the lethal amounts of zinc for nutrients containing

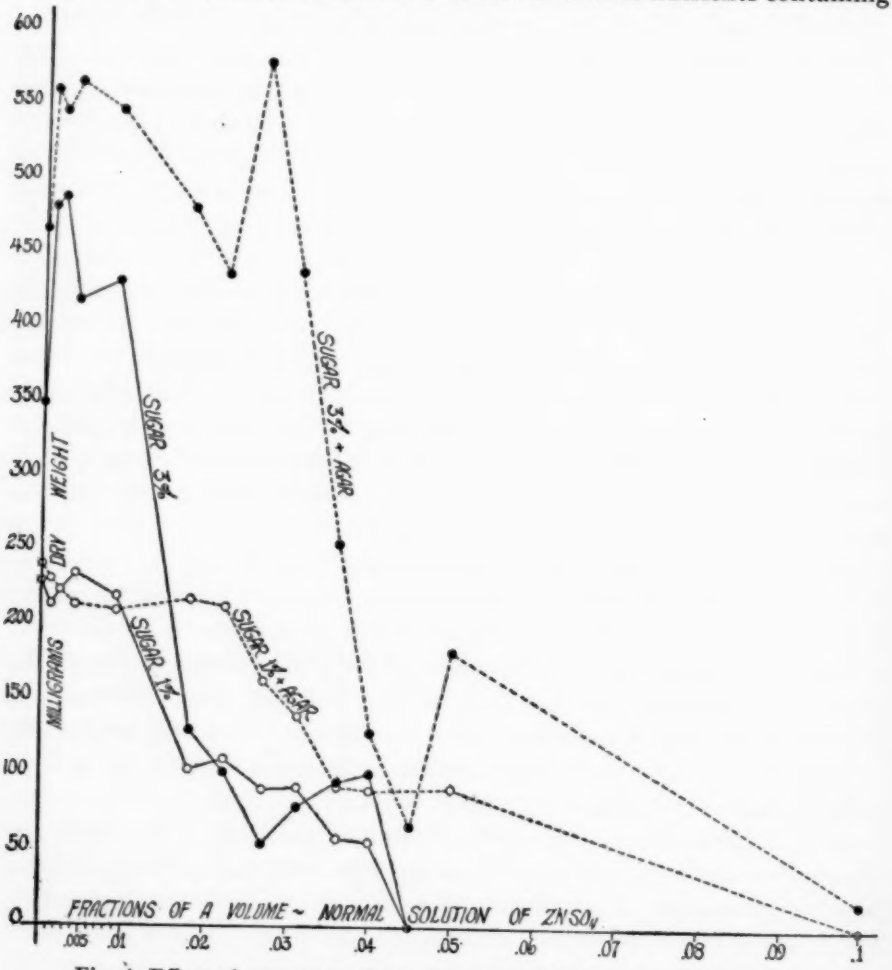

Fig. 4. Effect of agar upon the toxicity of $ZnSO_4$ for *Aspergillus niger*.

1 per cent and 3 per cent sugar, with and without agar. The data for this are shown graphically in figure 4.

With 1 per cent sugar solutions (table 10), the weight of fungus began to fall off from the high point, .004 N zinc sulphate. The loss in weight up to a concentration of .009 N zinc sulphate was very gradual, but from there on there was a definite decrease to some point betwen .04 N and

.045 N solutions, where there was no growth at all. The poisonous effect of the zinc began to be shown very definitely at .009 N concentrations where the felts were no longer complete, and the growth appeared in scattered patches. These scattered bits of growth were in some cases on the surface, in others, submerged. Between concentrations of .018 N and .045 N zinc sulphate there was still a decrease in mycelium weight but it was very gradual.

A striking difference is shown between the weights of mycelium using 1 per cent sugar nutrient and those where agar was also present (table 11). At a zinc sulphate concentration as high as .022 N the weight decreased only 11.7 per cent below that of the control, and only 8.2 per cent below that of a .001 N concentration. (It will be remembered that in these series the control weight was higher than any of the zinc culture weights, the next highest point being with a .001 N zinc sulphate solution.) The poisonous effect of the zinc here, as far as the completeness of the mycelial growth is concerned, did not begin until a concentration of .027 N was reached. From .022 N concentrations of the poison on, there was a steady, although gradual, decrease in weight of crop. At .045 N zinc sulphate, there was no longer any growth of *Aspergillus* with no colloid present; but when there was a colloid present there was some growth in two cases out of five even at a concentration of .1 N zinc sulphate. The average for the series was zero for this concentration, which may therefore be considered as lethal. Thus, with over twice as much zinc in the solution, when a colloid was present, *Aspergillus* was still able to grow.

The same reduction of the toxicity of the zinc salt held for 3 per cent sugar nutrient solutions containing agar. With no agar present there was a gradual decrease in weight from the high-point, .0003 N (table 12). The falling off was rather more abrupt between concentrations of .009 N and .018 N than was that for 1 per cent sugar alone, which may indicate that there was a starvation effect that influenced the poisonous action of zinc sulphate as well as its stimulative action. The same lethal point was found, however, for the 3 per cent sugar. Though the weight was considerably higher for 3 per cent than 1 per cent sugar cultures at .04 N concentrations of zinc sulphate, there was no growth at all at concentrations of .045 N. In this series the felts were first incomplete at zinc contents of .004 N, whereas with 1 per cent sugar this was not until .009 N in one case, and .018 N in three cases. This, too, seems to indicate the possibility of a 'starvation' effect which influenced the toxicity as well as the stimulating power of the zinc.

With the higher sugar content and agar (table 13) the felts were complete in some cases only until .018 N zinc concentrations were reached, but

the majority were complete up to concentrations of .036 N. The decrease in crop weight was gradual, beginning at the .004 N zinc sulphate solution, the high point for this series. The sudden increase in weight at .027 N zinc sulphate concentrations here seems rather abnormal. This might be explained on the ground that the agar which was in the medium may have been weighed with the felts, and notes not taken of it at the time, so that the higher weight may be due, at least in part, to dried agar. Whereas, with 3 per cent sugar alone, growth stopped at zinc concentrations between .04 N and .045 N, when agar was present there was still some slight growth at .1 N. The lethal point here, then, lay somewhere between .1 N and .2 N concentrations of zinc sulphate.

DISCUSSION OF RESULTS

Richards (1897) attempted to determine the effect of varying amounts of iron, zinc, cobalt, nickel, manganese, lithium, aluminium, and silicon salts and some organic substances, such as cocaine, morphine, amygdalin, antipyrin, and chloral hydrate on the growth of *Aspergillus niger*. He varied the nitrogen rather more than the carbohydrate source. Some series were set up, however, using glycerine with different nitrogen sources, and one substituting sodium acetate for sugar. His cultures were grown at 30°.

In all cases, Richards considered that abundant food was present. Glycerine, as a carbohydrate source gave a normal growth, he found, the conidia ripening, if anything, a little earlier than in sugar nutrient cultures. He did find, however, that on a poorer food substance, or by the addition of glycerine, though the mycelium developed more slowly, the rate of increase of growth due to the presence of zinc was almost the same as on a good food. His tables show an increase over the control of 155.3 per cent at the high-point (.0003 N zinc sulphate) in one series, and of 85.7 per cent at the high-point (.004 N zinc sulphate) in another. In two cases out of three, then, he found the proportional increase with zinc to be the same for sugar and glycerine nutrient solutions; in the other case, it was somewhat higher for the sugar solutions.

Watterson (1904) likewise reported that, though she got lower yields with glycerine as carbohydrate, the fungus responded with a decided increase in weight to the presence of zinc in the solution. Her figures show an increase of 144.3 per cent in one case, and of 102 per cent in another of dry weight of yield at a zinc concentration of .0005 N. Glycerine, therefore, though it appears to be a poorer food than 5 per cent sugar, cannot be compared to as low a sugar concentration even as 3 per cent.

The above results are contrary to those found in this work when very

low percentages of sugar were used. It does not seem possible, either, that stimulation would finally result in these 'starvation' cultures, for after four days there was an abundance of spores present with even the highest concentrations of zinc in the medium.

Asparagin in the medium, as a nitrogen source, according to Richards (1897), also did not affect the action of the zinc. He used another poor food stuff, sodium acetate. The growth here was slow, but there was nevertheless an increase with concentrations of .0003 N zinc sulphate of 83.3 per cent that of the control. The weights were very low here, a maximum of 55 mg. being obtained for this series. These results, then, do not agree with those obtained in this work with sugar percentages which yield as low a growth.

An explanation for the lack of response to zinc in those cultures where very low percentages of sugar were used is probably to be found in the greatly reduced vitality which must accompany growth on such a poor substratum. That this does not hold for spore production is evident from the very heavy fruiting obtained for all the cultures, with and without zinc, using sugar concentrations of .25 per cent and .5 per cent. This seems to be contrary to the view expressed by Richards (1910) that the ' . . . spore-forming process is one demanding a greater expenditure of energy than the mere vegetative growth of the hyphae.' On the other hand, deficiency of food, according to Pfeffer (1900) often induces the formation of spores. He also says, however, that if plants are too much starved, or growth almost suppressed by alkalis, acids, or poisons, the production of reproductive bodies almost or entirely ceases. Frank (1895), also, reported that with a lack of sufficient food, both the growth and fruiting of plants are below normal. Kiesel (1912) tested the effect of various acids on the germination of spores, the development of mycelium, and the production of spores. He concluded that all the acids tested had the same order as to potence for all three, so that one acid reducing the vegetative growth more than another should also reduce the formation of conidia more than the other.

It may be that with low sugar concentrations, mainly the toxic effects of the zinc show, as in those series where the control is the high point, or nearly the high point. This reaction to poor food supply may also account for the rather lower percentage increase due to the stimulative effect of zinc, obtained in this work for 3 per cent sugar, than has been found to occur by other workers who used mainly 5 per cent sugar. The vitality of the fungus when starved may be so low that it has not enough energy to show a response to the stimulant. Perhaps, when it is starved, the fungus can be said to have reached its greatest growth activity for the amount

of sugar present. In that case, the results obtained here might be compared to some obtained by Richards (1897) at quite the other extreme. When he had added iron to the medium in quantities which gave greatest growth for *Aspergillus niger*, he then found that no further increase in yield could be brought on by the addition of zinc sulphate. This he explained by saying that after the mycelium had reached the maximum of its growth activity, it could react to no more stimulation.

According to Richards (1910), 'The condition of the stimulated plant itself may cause a variation in the optimum concentration of the stimulant, as is shown by the effect of rise in temperature on the lowering of the toxic or stimulatory dose.' Here, it seems to me, we are unquestionably dealing with a peculiar condition of the fungus, brought about by insufficient food-supply.

Pfeffer (1900) states that ' . . . starvation may cause the reassimilation of metabolic products which under normal conditions remain permanently withdrawn from metabolism.' He also says that where a plant is stimulated by chemical elements 'increased growth appears to be due to a general power of reacting against injurious influences possessed by living organisms.' This power seems, paradoxically, from the results here obtained, to be increased, the weaker the condition of the fungus. He also says, in another place, that although deficiency of food diminishes the activity of growth and of respiration, it may cause the plant to work more economically.

Terroine, Trautmann, and Bonnet (1925) concluded that the metabolism of the cell is not modified by the concentration of food available. They measured the yield of energy produced (proportion of weight of crop to sugar used) and found that the concentration of nutrient elements might vary in the proportion of 1–15, and the utilization of energy for growth would remain the same. They grew *Sterigmatocystis nigra* (*Aspergillus niger*) in glucose solutions of 2 per cent, 10 per cent, 20 per cent, and 30 per cent. Zinc sulphate was present in all cases as a part of the culture medium.

Richards (1899) found that the economic coefficient of sugar was raised by the addition of zinc sulphate; in other words, there was a larger crop for a given amount of sugar when zinc was present in the solution. It would be interesting to determine what the result of a very poor food supply is on the economic coefficient of the sugar. According to Richards, one reason for the increased growth of the stimulated felts is the increased availability of the sugar. Why the zinc should not increase the availability of small amounts of sugar as well as of larger seems rather difficult to decide.

Pfeffer (1900) placed grape and cane sugar at the head of the list of carbohydrates of varying nutritive value for *Aspergillus niger* and *Penicillium glaucum* with ammonium nitrate as nitrogen source. He made the comment, however, that the nutritive value can be changed by temperature or concentration of the nutrient solution. The latter seems to be the case here, the change being, however, in the concentration of the sugar itself. According to him, 2–15 per cent grape or cane sugar makes a favorable medium for growth.

Aside from the results obtained using very low percentages of sugar, there is the question of the lack of response to zinc shown by the fungus when a colloidal solution was present in the medium. That this phenomenon is due to the colloidal nature of the substratum seems likely, because there was the same lack of stimulation shown with starch, a good energy source for *Aspergillus*, inulin, which also seems to be good as an energy source, and agar, which alone is a very poor food, but which inhibits response to zinc when present in the solution with sugar. It seems logical to account for this lack of response by assuming that the zinc in the solution is adsorbed by the disperse phase of the colloidal particles present.

This selective adsorption by colloids is a common enough occurrence. Hatschek (1916) in his *Introduction to the Physics and Chemistry of Colloids*, says: ' . . . various substances may not be adsorbed to the same extent, . . . or one or the other may be removed selectively, as, e.g., the coloring matter from sugar solutions.' In the case of charcoal and metallic salts in an aqueous solution, according to Taylor (1915), (in what he considers a pseudo-adsorption phenomenon) 'some salts of heavy metals are so completely removed from solution by it that not a trace of the metal can be detected in the liquid . . . but what has been taken out by the charcoal cannot be removed from it by washing with water.' No attempt was made here, to be sure, to find out whether any zinc was left in the solutions which had colloids present, or whether the starch, inulin, or agar had taken up the zinc. Taylor also says that in such cases the liquid then becomes strongly acid. If it were merely increased acidity which is responsible for stimulation, then, as Steinberg (1919) suggests, we should have greater instead of the same growth with a colloid present, due to this increased acidity of the medium. In the case of this work, no attempt was made to determine the acidity before or after the fungus had been growing in the medium.

That starch, too, removes substances from solution is reported by Lloyd (1911). To be sure, his results apply to various kinds of starches in the cold, but it would seem that they would also apply to the starch when 'dissolved.' He considers the loss of a neutral salt, like potassium

chloride, from solution, to be caused by adsorption of it by the starch.

Inorganic salts, acids, and bases are feebly adsorbed from water according to Taylor, but Carey (1923) has shown that an increase in acid concentration causes an increase in weight of acid adsorbed per gram of substance. This might hold good for adsorption of the metallic ion also from an acid solution. The culture medium is decidedly acid, and would be increasingly so the more zinc sulphate is added.

Long (1915) found that, with *Opuntia Blakeana* in a nutrient solution, there was practically always a slightly increased swelling over that in distilled water. His medium was neutral to begin with, but he does not say whether it remained neutral after the cactus had been in it.

The only reference to a similar inhibition of the poisonous effect of dissolved salts on plant tissues found was in a paper of Kahlenberg and True (1896). These workers were concerned with the poisonous effect of dissolved salts on roots of *Lupinus albus*. They attributed the lack of response to the poison to the lower toxicity of complex ions formed, though in each case the 'complex ions' might have been colloidal solutions. When copper salts were used, they concluded that the 'copper ion is more poisonous than a complex ion which contains copper.' Using dialyzed ferric chloride, which they said, contained no ferric or chloride ions, they found that the 'complex ions containing ferric iron in the dialyzed iron solution' were more poisonous than ferric ions of ferric chloride. The possibility of the effect being due to a colloid seems even more striking from their next results. They say that if dextrine is added first to a solution of mercuric chloride, precipitation of mercuric oxide by potassium hydroxide is prevented, so no mercuric ions are present. This solution is less poisonous than mercuric ions alone. They explain this on the ground that 'mercury has united with the dextrine to form a complex ion.' Is it not possible, however, that the colloidal dextrine adsorbs the mercuric ions from the solution? Their conclusion is: 'If the addition of certain substances to a solution containing a physiologically active ion forms a complex ion of much less powerful action, it follows that these additional ingredients afford a means of reducing, so to speak, the physiological action of the simple ion.' It would seem to me that the same conclusion could be drawn from the presence of colloids. Czapek (1913), in reviewing this paper, refers to the above effects as due to their colloidal nature, not mentioning the 'complex ion.' Pfeffer (1900) says in this connection, that ' . . . the presence of other substances may cause a soluble poison to be precipitated, or converted into an innocuous compound.'

The question of changes in permeability of the cell membrane due to the presence of a colloid, rather than the adsorption of the metallic ion by

the colloid might come into play here. Donnan and Harris (1911) have shown that when congo-red, a partial electrolyte, is present on one side of a parchment membrane, sodium chloride does not distribute itself in equal concentrations on both sides. In the opinion of Richards (1910), however, the different amounts of a substance necessary to produce stimulation is a question more of specific differences in the protoplasm itself, than of the permeability of the cell membranes.

On the whole, it seems to me that the adsorption by the colloid of the poison present is the best explanation for lack of response by *Aspergillus* under such circumstances to zinc sulphate. This might explain the fact that for years experimenters have been growing *Aspergillus niger* on slices of bread, far from 'physiologically clean,' and getting normal growth, although in order to get an 'unstimulated' growth in culture flasks, it is necessary to use purest chemicals and double-distilled water, and to have all culture flasks 'physiologically clean.'

The difference in lethal dose with and without a colloid present also seems an indication that the colloid can take up at least some of the zinc in the solution, if not all of it. Whether more agar in the solution would remove still more poison, and thus allow the fungus to grow at still higher zinc sulphate concentrations was not tested in this work. Since the amount of adsorption depends on the surface of the colloidal particles, this might be expected to be the case, at least for limited amounts. Depending on how the experiments were to be carried out; that is, were one to increase the amount of zinc sulphate rather than of agar, other factors would have to be considered, such as increased turgor pressure and acidity.

Richards (1897) found that *Aspergillus niger* was more susceptible to zinc than to any of the other metals he used. In a later paper (1899) he considered solutions of .002 N zinc sulphate concentration as just 'within the range of the toxic effect.' At this point the average weight of mycelium had dropped almost to the weight of the control felts. The economic coefficient of sugar also had fallen almost to that of the control. According to Kunkel (1913), zinc nitrate was the most toxic for *Monilia sitophila* of all the nitrates tested. In .000034 M solutions it completely inhibited the germination of spores when the fungus was grown in 5 per cent starch solutions. Latham (1905) found that chloroform behaved like heavy metals, causing stimulation in small quantities, and a cessation of growth in larger quantities. She found that germination was permanently stopped when concentrations of over 1 cc. per liter were used. With asparagine instead of ammonium nitrate in the nutrient, the sensitivity of *Aspergillus* to the poison was increased, development being prevented at concentrations of .67 cc. of chloroform per liter of solution. Kunkel (1913),

on the other hand, reported that when peptone was added to his starch media, the toxicity of ferric nitrate to *Monilia sitophila* was lessened.

From the series reported here with 1 per cent and 3 per cent sugar and no agar in the medium, it is forcibly brought out how resistant *Aspergillus niger* is to zinc sulphate in large quantities.

SUMMARY

A colloidal substratum inhibited the increased vegetative growth of *Aspergillus niger* usually found resulting from the addition of small amounts of zinc sulphate to the medium. A similar lack of stimulation also resulted when the carbohydrate source was present in low concentrations. The presence of a colloid in the nutrient solution also had an inhibiting effect upon the lethal doses of zinc sulphate for *Aspergillus niger*.

The above work was done in the Botanical Laboratory of Barnard College, Columbia University, under the direction of the late Professor Herbert M. Richards. The writer greatly appreciates his advice and interest.

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Chlorotylites, a fossil green alga from Alabama

MARSHALL A. HOWE

(WITH PLATE 15)

In November, 1931, Dean Edward W. Berry of The Johns Hopkins University, at Baltimore, sent to the writer a siliceous fossil from Alabama, together with a ground section thereof, in the belief that it represented an alga. A second specimen from the same locality was forwarded later, with the information that no more material was available, that both specimens had come from the Alabama Geological Survey, that the collection was made many years ago, and that name of collector and date of collection were unknown. It was further stated that the specimens came from Black Bluff on the Tombigbee River in Sumter County and from the Sucarnoche formation of the Middle Midway and that their general age was Lower Eocene. Professor Berry added, "Marine fossils are found in the outcrop, but there is also a bed of lignite near the top of the Bluff, which is obviously non-marine." Study of the fossils convinced the writer that organism was closely related to the recent genus *Chlorotylium*, the representatives of which inhabit fresh water and are found, for the most part, in rapidly flowing streams. They appear to be widely distributed, but are collected rather infrequently, presumably because they are normally heavily coated with lime and form small crusts that the average botanist passes by as something foreign to his field. Descriptions of genus and species follow:

Chlorotylites M. A. Howe, gen. nov.[1]

Class Chlorophyceae, family Chaetophoraceae. Thallus discoid, calcareous (or siliceous by replacement), consisting of compacted dichotomously branched fastigiate filaments, these composed of uniseriate cells of variable length, two

[1] *Chlorotylites* gen. nov. (fam. Chaetophoracearum, class. Chlorophycearum)* Thallus discoideus, calcareus (aut denique siliceus) e filis compactis dichotomis fastigiatis e cellulis uniseriatis in longitudine variabilibus, duobus aut tribus brevioribus cum una aut pluribus longioribus saepe alternantibus, compositus. Alga fossilis eocenica *Chlorotylio* Kuetz. recenti affinis.

Chlorotylites Berryi sp. nov. Thallo libero, disciformi (circ. 2½ cm. diam., 1 cm. crasso) in sectione lacunas paucas et lobos et convolutiones cerebriformes anastomosantes in verrucas aut mammillas stipatas superficiales 0.5–2 mm. latas apicibus erosis et lamellatis, desinentes monstrante; filis 8–13μ latis, cellulis 0.5–3-plo longioribus.

In loco "Black Bluff" dicto, in comitate "Sumter," Alabamae, Am.-Bor., in stratis geologicis inferiori-eocenicis.

or three shorter cells often alternating with one or more longer ones. Lower Eocene fossil, similar to recent *Chlorotylium* Kütz. in structure, but apparently unattached and more radial or discoid in mode of growth, with less regular development of long and short cells and less obvious lamellation. Type species, *Chlorotylites Berryi*.

Chlorotylites Berryi M. A. Howe, sp. nov. Thallus free, disciform (in the two specimens seen about 2 ½–5 cm. in diameter and 1 cm. thick) showing in section occasional lacunae and anastomosing cerebriform lobes and convolutions, ending in crowded superficial verrucae or mammillae mostly 0.5–2 mm. broad, the exposed apices often worn and lamellate; filaments 8–13μ broad, the cells mostly 0.5–3 times as long.

From Black Bluff, on Tombigbee River, Sumter Co., Alabama—Lower Eocene. Type specimen and section in the Herbarium of The New York Botanical Garden.

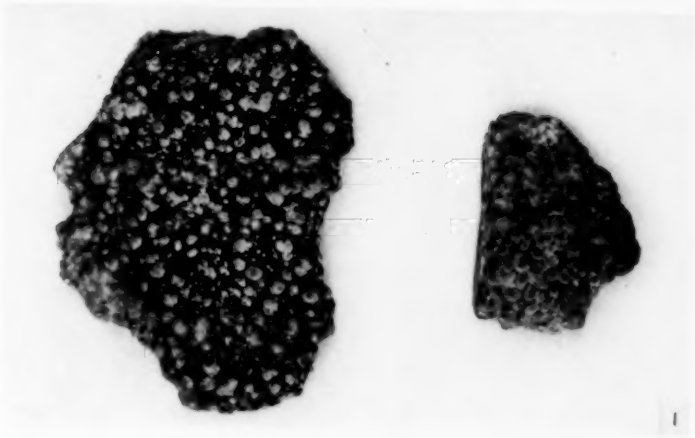
The species of the recent genus *Chlorotylium* form calcareous, often confluent hemispheric cushions on rocks, stones, wood, or leaves in freshwater ponds and streams, usually in rapidly flowing water. The specific name in *C. cataractarum*, the type and best-known species of the genus, sufficiently indicates its ordinary habitat. Of *Chlorotylites Berryi*, we have seen only two specimens. In the smaller one, of which our only ground section was made, the two faces of the disc are developed about equally; one could hardly say which was the upper surface and which the lower, but in the lower specimen there is a manifest differentiation of the two faces, the larger or shaded surface showing smaller and browner verrucae. The smaller pebble was apparently turned over frequently by the water-currents, so that it had no opportunity to develop dorsi-ventrality, while the larger heavier pebble was less often overturned. The one section made shows no trace of any original matrix on which the alga began its growth. This matrix was probably a leaf, a supposition that seems to explain both its disappearance and the flattened form of existing fossil. The fossil as found is siliceous, but it seems fair to assume that the original organism was calcareous, like its modern close relative.

THE NEW YORK BOTANICAL GARDEN

Explanation of plate 15

Chlorotylites Berryi

1. Photograph of the two specimens, natural size. The one at the right is the type.
2. A transverse ground section, enlarged somewhat less than two diameters (19/11).
3. A portion of the above section, showing the general surface at top, ×51.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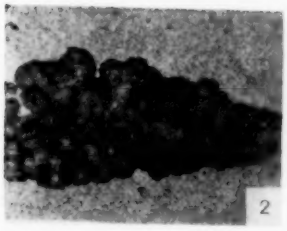
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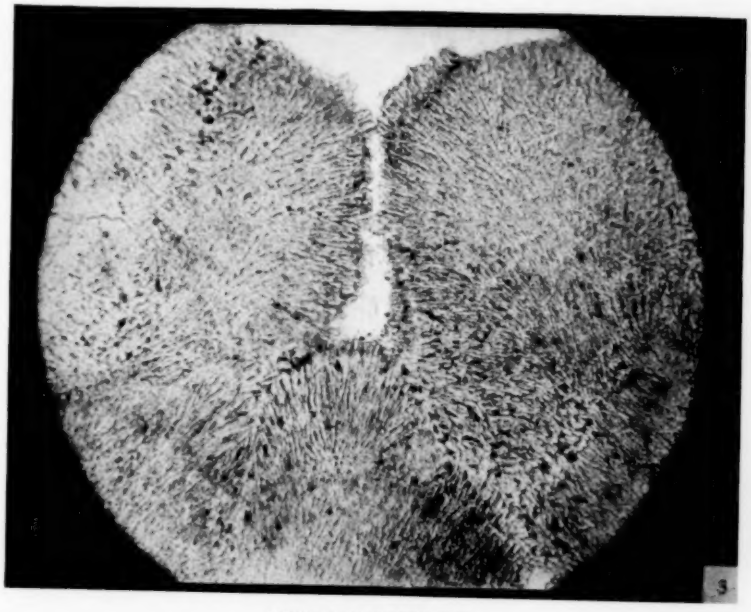
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A new ascocarpic species of Penicillium

MARJORIE E. SWIFT

(WITH A TEXT FIGURE)

The difficulties of understanding the natural relationships of species of fungi which have the penicillus type of asexual fructification and their relation to other ascomycetes are largely due to the rarity with which the perfect stages are produced in nature or in culture. It is well known that in other groups of ascomycetes similar types of asexual fructifications are sometimes connected with ascocarpic forms belonging to widely separated genera. Until some way is found to induce the formation of perithecia by the different types of *Penicillium*, their true relationships must remain a matter of conjecture. When, therefore, a species which forms ascocarps readily in culture is found, it becomes a subject of considerable interest to mycologists.

An unusual species recently appeared in our laboratory. It was one of three fungi isolated from a water-soaked spot on a Begonia leaf. It produces both the ascocarpic and conidial stages in culture, and its characters seem to be such as to warrant its being described as a new species.

Penicillium bacillosporum sp. nov.

Mycelio funiculoso, in variis substratis varie colorato, e.g., coloris pallide albulo-flavi in agaro maidis, coloris pallide rubidi in agaro dextroso. Substratis quoque coloratis, agaro maidis viridi, agaro dextroso rubro.

Conidiis 3–6×1–1.5μ, bacilliformibus, non numerosis; penicillo plerumque monoverticillato, sed interdum biverticillato; sterigmatibus 10–15×2.5–3.5μ; conidiophoris brevibus, 25–50×3–5μ; peritheciis 137–150μ, sub-albis vel alboflavis; ascis 10–12.5×7.5–10μ, globosis, ovoideis vel piriformibus, octosporis; sporidiis 3.5–4μ, globosis, tenuiter verrucosis.

Mycelium funiculose showing marked color differences on different nutrients, from pale yellow on corn meal agar to salmon on dextrose agar. Chromogenic effects in the medium itself also varied from bright green in corn meal agar to red in dextrose agar.[8]

Conidia 3–6×1.5μ, bacillar, not numerous, in long diverging thread-like chains, persistent at first, fragmenting in age. Penicillus predominantly monoverticillate, but occasionally biverticillate. Sterigmata 10–15×2.5–3.5μ, five or six in a whorl. Conidiophores short, 25–50×3–5μ, branched, formed mostly at right angles to the trailing sometimes funiculose hyphae.

Perithecia averaging 137–150μ in diameter, usually whitish to pale yellow, with thin but definite wall of interlacing hyphae. Asci 10–12.5×7.5–

10μ, globose, oval or pear-shaped, 8-spored. Ascospores 3.5–4μ, globose, finely verrucose, hyaline to yellowish in age.

On Begonia leaf, probably saprophytic.

Type locality, New York City.

Type culture and slide preparation deposited in the Herbarium of The New York Botanical Garden.

POSITION IN THOM'S GROUPS

According to Thom (1930), practically the only Penicillia which are known to produce ascocarps with any regularity belong to the *P. luteum-purpurogenum* group. Those reported in other groups are either doubtfully identified and described or have been found but once. No one except Schwartz (1927), for example, has ever been able to induce the sclerotia of *P. italicum* to develop asci; the ascocarp of *P. glaucum* described in detail by Brefeld (1874) has never been reported since, and, according to Thom (1930, p. 561), may have belonged to an *Aspergillus*. The strain of *P. candidum* described by Morini (1888) as producing perithecia has not been found again.

The characters of the *P. luteum-purpurogenum* group (Thom, 1915) in which the most common ascocarp-producing species fall are (1) a biverticillate type of conidial apparatus, (2) yellow color associated with aerial growth, and (3) yellow to red coloration in the medium. *P. bacillosporum* has a predominantly monoverticillate penicillus, although biverticillate individuals are occasionally found. The color of the aerial growth varies on different media from very pale yellow to bright salmon. The chromogenic effects in the medium range strikingly from bright clear green in corn meal agar to red in dextrose agar medium. The types of ascocarps and ascospores in the *P. luteum-purpurogenum* division, judging from descriptions, vary sufficiently to admit this species. The perithecium wall in this group ranges from the loosely constructed, easily crushed mass of hyphae typical of *P. Wortmanni* to the peridium of thick cells of *P. avellaneum* described by Thom and Turesson (1915), and the three-layer brittle wall of Lehman's (1920) *P. spiculisporum*. The perithecium of our *Penicillium* is easily crushed like that of *P. Wortmanni*, but its wall shows two rather distinct layers of compactly interwoven cells. The ascospores of the *P. luteum-purpurogenum* group range from the tricostate type of *P. luteum* to the echinulate and pitted types of *P. Wortmanni* and *P. avellaneum*. *P. bacillosporum* has ascospores with finely verrucose protuberances appearing as minute spines except under very high magnification where their wart-like form can be detected.

In view of these similarities and dissimilarities, the question as to whether or not *P. bacillosporum* belongs in the *P. luteum-purpurogenum*

group must be left open until further data on the ascocarp-forming species have made their classification more logical. Dr. Charles Thom, however, has examined cultures of this fungus and, despite the predominance of the monoverticillate conidial apparatus, states in a recent letter that he believes the shape of the sterigmata justifies its being placed "in the group specified by *P. spiculisporum* of Lehman" from which it "is properly separated by the conidium formation and the shape of the ascospores."

There are several characteristics of *P. bacillosporum* which make it distinctive as a species. The conidium is almost rod-shaped, with its length often as much as five times its width. These spores are produced very scantily on most media.[1] They can best be observed by examining a corn meal agar plate culture under low power. They occur in long thread-like diverging chains which break up in an angular fashion somewhat like chains of rectangular oidia. A second noticeable characteristic is the very prompt appearance on a variety of media of abundant, very pale yellowish perithecia, particularly marked on corn meal agar. Macroscopically the contrasting chromogenic effects produced in different media, to be described later, also mark the fungus as unusual.

SEXUALITY

Single spore culture work, with both conidia and ascospores, has shown *P. bacillosporum* to be homothallic. Twenty-seven single conidia and ten single ascospores were isolated and grown. Ascocarps appeared on corn meal agar within three days after a single spore of either type had been sown, provided the cultures were kept constantly at 28° C. Mature ascospores began to appear after twelve days.

Among the ascocarp-forming species of *Penicillium* studied to date the homothallic type seems to predominate. Schwartz (1927) concluded that *P. italicum* was homothallic after sclerotial bodies developed in cultures from single ascospores, these sclerotia being apparently identical with those from which he had obtained the ascospores by special treatment. Lehman (1920) does not state that he obtained ascospores from monospore cultures of *P. spiculisporum*. However, cultures from single spores of a strain of his species have been grown in this laboratory and they have produced ascocarps abundantly. *P. Wortmanni* and three undescribed species have also been tested for their sexuality and all produce ascocarps from single conidia as well as single ascospores.

[1] The terms "scanty" and "not abundant" in describing conidial production are used to contrast *P. bacillosporum* with the common imperfect forms of the Penicillia which produce spores in conspicuous powdery masses. The conidia are present, however, in numbers quite sufficient for diagnostic purposes.

The only case of heterothallism in the Penicillia thus far claimed to have been demonstrated was that of *P. luteum* (Zukal) Wehmer, reported by Derx (1926). He found that no ascocarps were formed in single spore cultures, but when certain cultures were paired fruit bodies developed. The possibility of other such cases is suggested by the fact that some ascogenous species have been said to lose their ability to form ascocarps after a period of culturing. The dying out or the accidental elimination of one of the unisexual strains might account for such an occurrence. Moreover, the abundance of "imperfect" species makes it feasible to suppose that these are unisexual strains which, if paired in the proper combinations, might produce their perithecial stage.

MORPHOLOGY

Conidia (fig. 1, a) occur in comparatively small numbers on most media. Their long divaricate tangled thread-like chains break up readily in old cultures, but are quite persistent when young, at which time the individual spore limits are often barely discernible. The spores are rod-like in form, 3–6×1–1.5μ, becoming slightly larger at one end with maturity, and in very old cultures approaching an oval contour. The terminal spore of the chain is consistently shorter than the others and nearly oval. Upon germination the spore swells to approximately twice its width, but not increasing appreciably in length, and sends out one or two germ tubes which quickly begin to septate and branch. The appearance of the young colony differs greatly on different media. On corn meal agar the mycelium is almost entirely submerged and hyaline until the very pale yellow ascocarps begin to appear (after three days) at which time the rather scattered conidial branches also develop on trailing, sometimes funiculose, hyphae, and the green color begins to appear in the medium.

The sterigmata occur usually in one verticil, five or six in the whorl (fig. 1, a). They are usually acuminate to lanceolate, with their form varying, however, depending upon age and fertility. One short side branch frequently occurs, suggesting a biverticillate condition, or two or three shorter branches may form a true verticil of metulae. This definitely biverticillate penicillus, however, is not common.

The conidiophores are short, usually extending at right angles from the main hyphae. Branching of the fertile hyphae is common. The walls of the conidiophore sometimes show a slight roughening.

Ascocarps are approximately globose, non-ostiolate, averaging 137–150μ in diameter, and surrounded by a loose mat of branching roughened hyphae usually very pale yellowish. This weft of mycelium causes considerable difficulty in cytological study by enclosing the fruit body in a

film of air which prevents good fixation. The wall of the perithecium (fig. 1, e) is composed of interlacing, quite closely compacted, hyphae in two more or less distinct layers, the outer about 5–7.5μ thick, darkly-staining, and merging gradually into the inner which is composed of more lightly-staining larger cells.

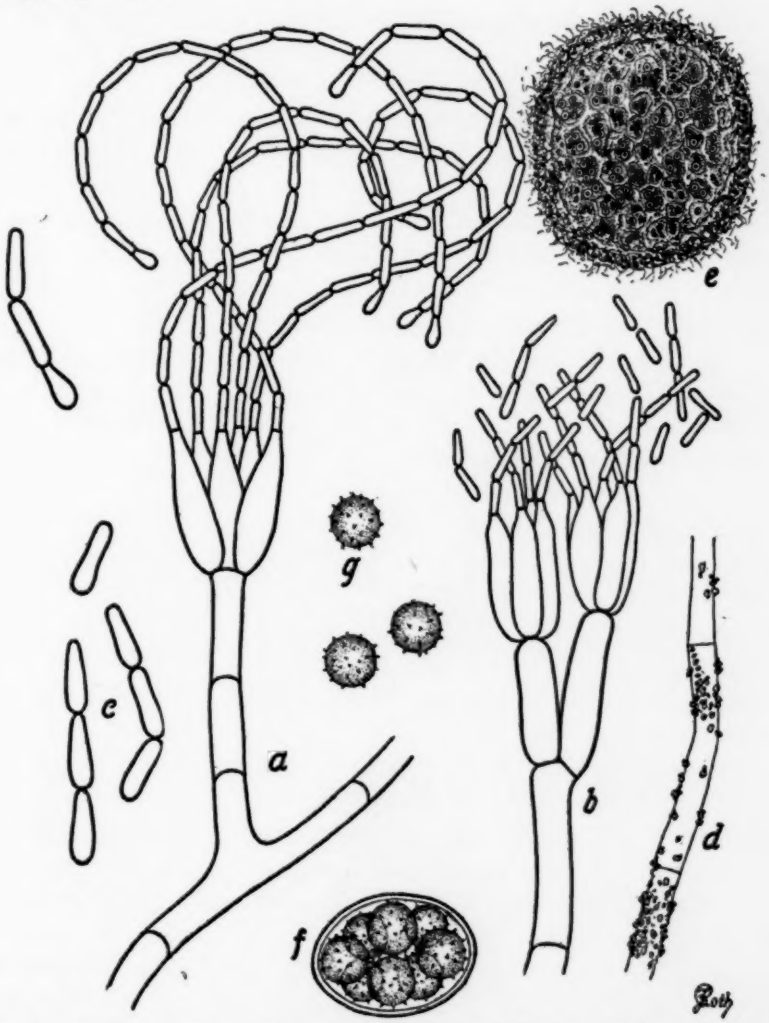

Fig. 1. *Penicillium bacillosporum.* (a) Typical conidiophore with spore chains. (b) Branched conidiophore. (c) Individual conidia. (d) Hypha with yellow granules. (e) Section of ascocarp showing asci and two-layer wall. (f) Ascus with ascospores. (g) Individual ascospores.

The asci (fig. 1, f) are spherical, oval or pear-shaped, 10–12.5$\times$7.5–10μ. They begin to show mature ascospores within twelve days on corn meal

agar at 28° C. The ascospores (fig. 1, g) are spherical, 3.5–4μ and very finely verrucose. They are at first hyaline, but in age show an orange yellow tint. Upon germination they swell to about twice their normal size and send out one or two germ tubes.

The mycelium is hyaline, branching, septate, 2.5–4μ, and often roughened with yellowish deposits particularly about the perithecia.

CULTURE CHARACTERISTICS

The various color differences both in the medium and in the fungus mycelium on different agars is, as above noted, one of the interesting features of this species.

Corn meal agar (pH 6.8). Little aerial growth except about the very pale yellowish (18 C 1, Maerz and Paul, 1930) wefted ascocarps which dot the surface of the colony in a few days. Submerged hyphae hyaline. Reverse and medium becoming in certain areas clear bright to deep green (21 L 9 to 24 A 12). Conidia few and produced on trailing ropy hyphae chiefly near the ascocarps.

Czapek agar (pH 5.0). Growth somewhat restricted; velvety aerial felt more or less buckled. Colonies pale yellow (18 G 1) throughout with slight green tinge at extreme center; later becoming sulphur yellow (10 J 1) in the center with orange (10 K 8) droplets. Reverse at first dark and indefinite, becoming green at edges of colony (5 G 9). Both ascocarps and conidia present, but latter scant.

Potato dextrose agar (pH 4.5). Thick aerial growth, powdery to loosely velvety, persistently and uniformly pale yellow (18 F 1) with faint suggestion of green at center. Reverse dull red (6 K 11). Ascocarps and conidia comparatively abundant.

Dextrose agar (pH 4.5). Thick aerial growth, powdery to loosely velvety. At first pale yellow (18 H 1) with narrow white margin, becoming deep salmon (1 A and B 11) with deeper-colored drops (1 H 12). Reverse at first deep green (25 J 3), becoming dull red except at edges where green persists. Medium red (9 G 11). Conidia and ascocarps comparatively numerous.

Sabouraud agar (pH 4.4). Growth coarsely velvety. At first green then center area dull reddish gray (6 B 9) surrounded by band of greenish gray (29 Z 9), margin white. Reverse red (6 L 10). Ascocarps not visible macroscopically, and present only in small numbers. Conidia quite numerous.

Potato plugs. Growth irregularly velvety, dull gray green (29 Z 3) becoming yellowish (16 D 1), with margin white. Numerous conidia but few ascocarps.

The extreme range from green to red color produced in the media was

thought to be due possibly to differences in hydrogen ion concentration, the green predominating in lower, the red in higher acidities. Accordingly, transfers were made to two tubes of corn meal agar, one with the usual pH of 6.8, the other changed to 4.8. At the same time transfers were made to two tubes of potato dextrose agar, one with the usual pH of 4.5, the other changed to 7.0. After two months of growth, no differences in color production or in rate of growth have been observed.

The local and more or less spasmodic production of the bright green pigment in corn meal agar was found not to be correlated with temperature. Cultures grown at 28° C. and 40° C. showed similar coloration, and very little difference in rate of growth. The perithecia produced at the high temperature were somewhat smaller and matured ascospores at a later date than those grown at 28° C. At 52° C. no growth of the fungus occurred.

This work has been done under the supervision of Dr. B. O. Dodge, whose valuable guidance is acknowledged with appreciation. I am also indebted to Dr. Charles Thom for his courtesy in examining cultures of *P. bacillosporum* and criticising the manuscript.

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INDEX TO AMERICAN BOTANICAL LITERATURE

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